

WO0125466

Publication Title:

PRODUCER CELL FOR THE PRODUCTION OF RETROVIRAL VECTORS

Abstract:

A method is provided for modifying a producer cell which producer cell comprises integrated into its genome a provirus which provirus comprises one or more recombinase recognition sequences within or upstream of its 3' LTR, the method comprising: introducing into the cell a construct comprising a 5' recombinase recognition sequence, an LTR and a 3' recombinase recognition sequence in that order, in the presence of a recombinase which is capable of acting on the recombinase recognition site(s) such that the nucleotide sequence between the 5' and 3' recombinase recognition sequences in the construct is introduced into the provirus.

Data supplied from the esp@cenet database - <http://ep.espacenet.com>

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number
WO 01/25466 A1

(51) International Patent Classification⁷: **C12N 15/867**,
15/90, 5/10, 7/01, A61K 48/00

(UK) Limited, Medawar Centre, Robert Robinson Avenue,
The Oxford Science Park, Oxford OX4 4GA (GB).

(21) International Application Number: PCT/GB00/03837

(74) Agents: **HARDING, Charles, Thomas** et al.; D Young &
Co., 21 New Fetter Lane, London EC4A 1DA (GB).

(22) International Filing Date: 5 October 2000 (05.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9923558.2 5 October 1999 (05.10.1999) GB

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **SLINGSBY, Jason**
[GB/GB]; 91 Lacy Road, Putney, London SW15 1NR
(GB). **KINGSMAN, Susan, Mary** [GB/GB]; Oxford
BioMedica (UK) Limited, Medawar Centre, Robert
Robinson Avenue, The Oxford Science Park, Oxford
OX4 4GA (GB). **ROHLL, Jonathan** [GB/GB]; Oxford
BioMedica (UK) Limited, Medawar Centre, Robert Robin-
son Avenue, The Oxford Science Park, Oxford OX4 4GA
(GB). **SLADE, Andrew** [GB/GB]; Oxford BioMedica

Published:

- With international search report.
- Before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 01/25466 A1

(54) Title: PRODUCER CELL FOR THE PRODUCTION OF RETROVIRAL VECTORS

(57) Abstract: A method is provided for modifying a producer cell which producer cell comprises integrated into its genome a provirus which provirus comprises one or more recombinase recognition sequences within or upstream of its 3' LTR, the method comprising: introducing into the cell a construct comprising a 5' recombinase recognition sequence, an LTR and a 3' recombinase recognition sequence in that order, in the presence of a recombinase which is capable of acting on the recombinase recognition site(s) such that the nucleotide sequence between the 5' and 3' recombinase recognition sequences in the construct is introduced into the provirus.

PRODUCER CELL FOR THE PRODUCTION OF RETROVIRAL VECTORS

FIELD OF THE INVENTION

5

The present invention relates to retroviral vectors, in particular to high titre regulatable retroviral vectors.

BACKGROUND TO THE INVENTION

10

Retroviruses have been proposed as a delivery system (otherwise expressed as a delivery vehicle or delivery vector) for *inter alia* the transfer of a nucleotide sequence of interest (NOI), or a plurality of NOIs, to one or more sites of interest. The transfer can occur *in vitro*, *ex vivo*, *in vivo*, or combinations thereof. When used in this fashion, the retroviruses are typically called retroviral vectors or recombinant retroviral vectors. Retroviral vectors have been exploited to study various aspects of the retrovirus life cycle, including receptor usage, reverse transcription and RNA packaging (reviewed by Miller, 1992 Curr Top Microbiol Immunol 158:1-24).

15

In a typical recombinant retroviral vector for use in gene therapy, at least part of one or more of the *gag*, *pol* and *env* protein coding regions may be removed from the virus. This makes the retroviral vector replication-defective. The removed portions may even be replaced by a NOI in order to generate a virus capable of integrating its genome into a host genome but wherein the modified viral genome is unable to propagate itself due to a lack of structural proteins. When integrated in the host genome, expression of the NOI occurs - resulting in, for example, a therapeutic effect. Thus, the transfer of a NOI into a site of interest is typically achieved by: integrating the NOI into the recombinant viral vector; packaging the modified viral vector into a virion coat; and allowing transduction of a site of interest - such as a targetted cell or a targetted cell population.

25

30

It is possible to propagate and isolate quantities of retroviral vectors (e.g. to prepare suitable titres of the retroviral vector) for subsequent transduction of, for example, a site of interest by using a combination of a packaging or helper cell line and a recombinant vector.

-2-

In some instances, propagation and isolation may entail isolation of the retroviral *gag*, *pol* and *env* genes and their separate introduction into a host cell to produce a "packaging cell line". The packaging cell line produces the proteins required for packaging retroviral RNA but it does not produce RNA-containing retroviral vectors. However, when a recombinant vector carrying a NOI and a *psi* region is introduced into the packaging cell line, the helper proteins can package the *psi*-positive recombinant vector to produce the recombinant virus stock. This can be used to infect cells to introduce the NOI into the genome of the cells. The recombinant virus whose genome lacks all genes required to make viral proteins can infect only once and cannot propagate. Hence, the NOI is introduced into the host cell genome without the generation of potentially harmful retrovirus. A summary of the available packaging lines is presented in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 449).

Retroviral packaging cell lines have been developed to produce retroviral vectors. These cell lines are designed to express three components, which may be located on three separate expression constructs. The *gag/pol* expression construct encodes structural and enzymatic components required in particle formation, maturation, reverse transcription and integration. The envelope (*env*) construct expresses a retroviral or non-retroviral envelope protein, which mediates viral entry into cells by binding to its cognate receptor. The third expression construct produces the retroviral RNA genome containing a *psi* region, which is packaged into mature, enveloped retroviral particles.

It has been observed that different methods, such as electroporation, transfection and retroviral transduction, which have been used to introduce the retroviral expression construct for the RNA genome, termed "the genome", into packaging cells produce different results. These different results can include different end points or "yield" of retroviral producer lines resulting from the derived cell lines. Moreover, electroporation and transfection methods can be problematic in the sense that the titre levels are not always at a satisfactory level.

By way of example, the transfection of a plasmid DNA construct into packaging cells from a MLV packaging cell line of human origin, called FLYA13, yielded low retroviral

-3-

vector titres even when different transfection reagents such as calcium phosphate precipitation and fugene transfection reagent were used. The average titres from selected stably transfected cell lines clones ranged from about 10^3 to about 10^4 per ml. In addition, clones generated by electroporation of constructs gave similar titres of from about 10^3 to about 10^4 per ml with no clones identified producing at $>10^5$ per ml. However, when MLV vector particles are prepared in a transient transfection system with a different envelope pseudotype to the packaging cell, and used to transduce a retroviral packaging cell, stably transduced cell lines made by this transduction method produce retrovirus at 10^6 to 10^7 per ml. Therefore, these results suggest that retroviral transduction is a preferred method for genome introduction into packaging cell lines in order to generate high titre producer cell lines. However, when retroviral transduction is used to introduce a regulated/inactivated retroviral vector genome into packaging cell lines, the regulated retroviral vectors may not be produced in sufficient quantities from these cell lines.

By way of example, some retroviral vectors may comprise (i) internal expression constructs which are themselves regulated or (ii) regulated elements which are present in retroviral 3' LTR sequences, either by design or by their nature. Examples of these regulated vectors include but are not limited to hypoxic regulated vectors and self inactivating (SIN) vectors. If transduced producer cell lines are generated with these regulated vectors, the regulated or inactivated 3' U3 sequence of the LTR is copied to the 5' LTR by the process of retroviral reverse transcription and integration. Therefore, in the producer cell line, the 5' U3 promoter sequence directing expression of retroviral RNA genomes is identical to the regulated or inactivated 3' U3 promoter. This will result in very low levels of retroviral genome production and consequently low titres of functional retrovirus vectors being produced.

One example of such a regulated retroviral system includes MLV and lentivirus vector constructs where the 3' retroviral U3 enhancer element is replaced with a hypoxia responsive element (HRE) or other physiologically regulated, tumour specific or tissue-specific promoters. When these vectors are used to make a transduced producer cell line, the 3' U3 sequence containing the HRE element is copied to the 5' LTR position and retroviral genomes will only be produced under hypoxic conditions or chemical mimics

-4-

of hypoxia, such as heavy metal ions and desferrioxamine. Such a requirement for "induction for retroviral production" is not preferable as the different hypoxia induction protocols negatively affect retroviral producer cell viability.

- 5 By way of further example, lentivector U3 enhancers are dependent on the transactivator TAT for transcriptional activation. Therefore, a lentivector producer cell line generated by transduction requires the presence of TAT for high level expression of the lentivector genome construct. The expression of TAT is not preferable in such a packaging cell line and therefore, in the absence of TAT, only very low titres will be produced from
10 transduced producer cells generated in this way.

Another example of a regulated retroviral systems includes MLV or lentivirus self-inactivating (SIN) vectors. These vectors contain deletions of the elements in their 3' U3 sequences responsible for transcriptional activity. Therefore, on transduction of target
15 cells, the transcriptionally inactive 3' U3 sequence is copied to the 5' LTR position. In standard configurations, an internal expression cassette directs therapeutic or marker gene expression. However, if SIN vectors are used to make a transduced retroviral producer line, there will be no transcriptional elements present to direct high levels of retroviral RNA genome expression.

20

Although it is possible to carry out retroviral transduction with much lower-titre vector stocks, for practical reasons, high-titre retrovirus is desirable, especially when a large number of cells must be infected. In addition, high titres are a requirement for transduction of a large percentage of certain cell types. For example, the frequency of
25 human hematopoietic progenitor cell infection is strongly dependent on vector titre, and useful frequencies of infection occur only with very high-titre stocks (Hock and Miller 1986 Nature 320: 275-277; Hogge and Humphries 1987 Blood 69: 611-617). In these cases, it is not sufficient simply to expose the cells to a larger volume of virus to compensate for a low virus titre. On the contrary, in some cases, the concentration of
30 infectious vector virions may be critical to promote efficient transduction.

SUMMARY OF THE INVENTION

We have now shown that it is possible to obtain transduced producer cells capable of producing high titre regulated retroviral vectors by replacing at least the 3'LTR of the integrated provirus using a recombinase based system. Thus whereas with the prior art, the U3 region of the 3'LTR is the same as that of the U3 region of the 5' LTR (and vice versa for the U5 region) in the provirus due to the way in which the viral vector integrates, the introduction of, for example, a replacement 3'LTR results in a provirus that has a 5'LTR and a 3'LTR that differ. The packaged viral vectors produced by transcription of the proviral genome within the producer cells may then ultimately be used to transduce target cells where the regulatable sequences present in the 3'LTR of the provirus in the producer cells are then present in the 5'LTR of the provirus in the target cells and consequently regulate transcription from the provirus as required.

This allows the introduction of a 3'LTR, for example a regulatable 3'LTR, into the provirus that was not desirable in the original viral vector used to transduce the producer cells since the consequential appearance of the regulatable 3'LTR U3 sequences in the 5'LTR in the provirus may lead to a reduced viral titre.

Consequently, the present invention allows transduced producer cells to be constructed that are capable of producing high titre regulated retroviral vectors by virtue of comprising a 5'LTR that directs high level expression of the viral genome in the producer cell and a different 3'LTR which as a result of the process of integration into a target cell will then result in a provirus in the target cell genome that exhibits regulatable expression.

In particular, the present invention allows the modification of a provirus integrated into the genome of the producer cells that have been selected for their high titre virus production such that the resulting packaged viral particles produced from the provirus may be used to transduce target cells resulting in a provirus integrated into the genome of the target cells that has a different, and preferably regulatable 5'LTR to that of the producer cell provirus.

-6-

The present invention is not limited to replacement of the 3'LTR of the provirus in the high titre producer cells, but may also include replacement of the 5'LTR and other viral sequences and/or the introduction of NOIs by the use of suitable constructs, as shown in the Figures.

5

Accordingly, the present invention provides a method of modifying a producer cell which producer cell comprises integrated into its genome a provirus which provirus comprises one or more recombinase recognition sequences within or upstream of its 3' LTR, the method comprising: introducing into the cell a construct comprising a 5' recombinase
10 recognition sequence, an LTR and a 3' recombinase recognition sequence in that order, in the presence of a recombinase which is capable of acting on the recombinase recognition site(s) such that the nucleotide sequence between the 5' and 3' recombinase recognition sequences in the construct is introduced into the provirus.

15 Preferably the LTR is a heterologous regulatable LTR.

The present invention further provides a nucleic acid vector comprising a 5' recombinase recognition sequence, a regulatable LTR and a 3' recombinase recognition sequence in that order.

20

In any of the above aspects and embodiments of the invention, preferably the construct, nucleic acid molecule and/or nucleic acid vector further comprises at least one NOI between the 5' recombinase recognition sequence and the regulatable LTR.

25 Preferably the construct, nucleic acid molecule and/or nucleic acid vector further comprises a 5'LTR and/or a packaging signal

In one embodiment of the invention, the LTR is inactive/transcriptionally quiescent.

30 The construct, nucleic acid molecule and/or nucleic acid vector of the invention may be used in a recombinase assisted method to introduce a regulated LTR into a proviral genome integrated into a producer cell genome.

-7-

The present invention also provides a producer cell obtainable by the method of the invention, preferably a high titre producer cells. Also provided is an infectious retroviral particle obtained by the above method.

- 5 The present invention further provides a high titre producer cell comprising integrated into its genome a provirus, which provirus comprises a recombinase recognition site, a 5' LTR and a 3'LTR which 3'LTR differs from the 5'LTR. Such a producer cell will typically have been produced by the method of the invention.
- 10 Preferably the 5'LTR and the 3'LTR referred to for the purposes of comparison are both "active". The term "active" within the present context means transcriptionally active, that is to say, the 5'LTR comprises a promoter that directs transcription of the viral genome and the 3'LTR comprises a transcriptional stop sequence to terminate transcription. This distinction is relevant since if a provirus produced by the method of the invention
- 15 comprises more than one 5' LTR or 3'LTR, at least one but not all must be active to allow viral production. Further, if the provirus comprises more than one 3'LTR then it is generally the upstream one that will be active since transcription will tend not to read through to the downstream 3' LTR.
- 20 In addition, where the method of the invention results in an insertion of a 3'LTR upstream of the original 3'LTR, the comparison should be performed between the additional 3'LTR and the original 5'LTR and not the two original LTRs. Thus it is permitted to have a 5'LTR and 3'LTR within the same provirus that are the same provided that there is also a 5'LTR and 3'LTR that differ.
- 25 In another aspect, the present invention provides a derived producer cell comprising integrated into its genome a retroviral vector comprising in the 5' to 3' direction a first 5' LTR; a second NOI operably linked to a second regulatable 3' LTR; and a third 3'LTR; wherein the third 3'LTR is positioned downstream of the second regulatable 3'LTR in the
- 30 producer cell.

-8-

Preferably the first 5' LTR comprising 5'R and 5' U5 sequences is derivable from a first vector; the second NOI operably linked to a second regulatable 3' LTR is derivable from a second vector; and the third 3'LTR is derivable from the first vector.

- 5 In a preferred embodiment, the first vector further comprises an internal LTR located upstream of the first NOI and downstream of the packaging signal wherein the internal LTR comprises a heterologous U3 sequence linked to heterologous R and U5 sequences.

Preferably the heterologous R and U5 sequences are lentiviral derivable R and U5
10 sequences, such as ELAV R and U5 sequences.

In a further preferred embodiment, the third 3'LTR is transcriptionally active but expression is directed away from the second regulatable 3'LTR.

- 15 In another embodiment, the second vector comprises a second NOI operably linked to a second regulatable 3'LTR comprising at least one recombinase recognition sequence. Preferably the second regulatable 3'LTR comprises a deletion in the U3 sequences in the 3'LTR.

- 20 Preferably, the second NOI comprises a discistronic construct, more preferably a discistronic construct comprising a therapeutic gene, an internal ribosomal entry site (IRES) and a reporter gene.

The present invention further provides in another embodiment, a method for producing a
25 high titre regulatable retroviral vector, the method comprising the steps of:

- (i) providing a derived producer cell comprising integrated into its genome a first vector;
(ii) introducing a second vector into the derived producer cell using a recombinase assisted method; wherein the derived producer cell comprises a retroviral vector comprising in the 5' to 3' direction a first 5' LTR; a second NOI operably linked to a
30 second regulatable 3' LTR; and a third 3'LTR; wherein the third 3'LTR is positioned downstream of the second regulatable 3'LTR in the derived producer cell.

-9-

The present invention also provides the use of a recombinase assisted mechanism to introduce a regulated 3'LTR into a derived producer cell line to produce a high titre regulated retroviral vector.

- 5 Aspects of the present invention are also presented in the accompanying claims and in the following description and discussion.

These aspects are presented under separate section headings. However, it is to be understood that the teachings under each section heading are not necessarily limited to
10 that particular section heading.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is advantageous because:

15

(i) it enables regulated retroviral vectors to be produced at high titres from transduced producer cell lines.

20

(ii) it removes the uncertainty associated with the process of producer cell line derivation and the necessity to screen large numbers of producer cell lines each time a new retroviral expression construct is introduced into a producer cell line.

25

(iii) it greatly facilitates the generation of high titre retroviral stocks without the use of marker genes (such as but not limited to β -galactosidase, green fluorescent protein) and antibiotic resistance genes.

30

(iv) it avoids the derivation of low titre transfected producer cell lines or the use of hypoxic conditions or chemical mimics for production from traditionally derived transduced producer lines.

(v) it enables the production of SIN vectors by stable cell line producer technology. Previously, SIN vectors have not been amenable to production by stable cell line producer technology because the deletion of the 3'U3 sequence resulted in at least a tenfold lower

-10-

titre of self-inactivating (SIN) vectors in comparison with vectors having intact LTRs. Consequently, SIN vectors have had to be prepared using transfection-based transient expression systems.

5 PRODUCER CELL

The high titre regulated retroviral vector particles of the present invention are typically generated in a suitable producer cell. Producer cells are generally mammalian cells but can be, for example, insect cells. A producer cell may be a packaging cell containing the virus structural genes, normally integrated into its genome into which the regulated retroviral vectors of the present invention are introduced. Alternatively the producer cell may be transfected with nucleic acid sequences encoding structural components, such as *gag/pol/env* on one or more vectors such as plasmids, adenovirus vectors, herpes viral vectors or any method known to deliver functional DNA into target cells. The vectors according to the present invention are then introduced into the packaging cell by the methods of the present invention.

As used herein, the term "producer cell" or "vector producing cell" refers to a cell which contains all the elements necessary for production of regulated retroviral vector particles and regulated retroviral delivery systems.

Preferably, the producer cell is obtainable from a stable producer cell line.

Preferably, the producer cell is obtainable from a derived stable producer cell line.

Preferably, the producer cell is obtainable from a derived producer cell line

As used herein, the term "derived producer cell line" is a transduced producer cell line which has been screened and selected for high expression of a marker gene. Such cell lines contain retroviral insertions in integration sites that support high level expression from the retroviral genome. The term "derived producer cell line" is used interchangeably with the term "derived stable producer cell line" and the term "stable producer cell line"

-11-

Preferably the derived producer cell line includes but is not limited to a retroviral and/or a lentiviral producer cell.

Preferably the derived producer cell line is an HIV or EIAV producer cell line, more preferably an EIAV producer cell line.

Preferably the envelope protein sequences, and nucleocapsid sequences are all stably integrated in the producer and/or packaging cell. However, one or more of these sequences could also exist in episomal form and gene expression could occur from the episome.

PACKAGING CELL

As used herein, the term "packaging cell" refers to a cell which contains those elements necessary for production of infectious recombinant virus which are lacking in a recombinant viral vector. Typically, such packaging cells contain one or more expression cassettes which are capable of expressing viral structural proteins (such as *gag*, *pol* and *env*) but they do not contain a packaging signal.

The term "packaging signal" which is referred to interchangeably as "packaging sequence" or "*psi*" is used in reference to the non-coding sequence required for encapsidation of retroviral RNA strands during viral particle formation.

Packaging cell lines suitable for use with the above-described vector constructs may be readily prepared (see also WO 92/05266), and utilised to create producer cell lines for the production of retroviral vector particles. As already mentioned, a summary of the available packaging lines is presented in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 449).

The packaging cell lines are useful for providing the gene products necessary to encapsidate and provide a membrane protein for a high titre regulated retrovirus vector and regulated nucleic gene delivery vehicle production. When regulated retrovirus sequences are introduced into the packaging cell lines, such sequences are encapsidated

-12-

with the nucleocapsid (*gag/pol*) proteins and these units then bud through the cell membrane to become surrounded in cell membrane and to contain the envelope protein produced in the packaging cell line. These infectious regulated retroviruses are useful as infectious units *per se* or as gene delivery vectors.

5

The packaging cell may be a cell cultured *in vitro* such as a tissue culture cell line. Suitable cell lines include but are not limited to mammalian cells such as murine fibroblast derived cell lines or human cell lines. Preferably the packaging cell line is a human cell line, such as for example: HEK293, 293-T, TE671, HT1080.

10

Alternatively, the packaging cell may be a cell derived from the individual to be treated such as a monocyte, macrophage, blood cell or fibroblast. The cell may be isolated from an individual and the packaging and vector components administered *ex vivo* followed by re-administration of the autologous packaging cells.

15

Methods for introducing retroviral packaging and vector components into packaging/producer cells are described in the present invention.

Preferably the method of the present invention utilises a recombinase assisted mechanism.

20

Preferably the method of the present invention utilises a recombinase assisted mechanism which facilitates the production of high titre regulated retroviral vectors from the producer cells of the present invention.

25 RECOMBINASE ASSISTED MECHANISM

As used herein, the term "recombinase assisted system" includes but is not limited to a system using the Cre recombinase / loxP recognition sites of bacteriophage P1 or the site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between
30 34 bp FLP recognition targets (FRTs).

The site-specific FLP recombinase of *S. cerevisiae* which catalyses recombination events between 34 bp FLP recognition targets (FRTs) has been configured into DNA constructs

-13-

in order to generate high level producer cell lines using recombinase-assisted recombination events (Karreman *et al.* (1996) NAR 24, 1616-1624). A similar system has been developed using the Cre recombinase / loxP recognition sites of bacteriophage P1. This was configured into a retroviral genome such that high titre retroviral producer cell lines were generated (Vanin *et al.* (1997) J Virol 71, 7820-7826). However, the use of the second method (Vanin *et al. ibid*) has centered around the exchange of the central portions of a retroviral cassette using a recombinase-assisted system. Moreover, these methods have used genes encoding selectable markers such as neo^R and puro^R (Vanin *et al. ibid*) and luciferase and puro^R linked by an IRES sequence (Karreman *et al. ibid*).

10 Karreman and Vanin do not demonstrate or suggest that: (i) a regulated or inactive 3'U3 sequence of the 3'LTR can be introduced into a producer cell via a recombinase-assisted mechanism or (ii) that therapeutic genes under the control of a regulated LTR may be introduced into a producer cell line via a recombinase assisted step. Vanin *et al. ibid* suggests that his Cre-mediated recombination approach to retroviral producer cell line production may be used in combination with other modifications which should result in improved vector performance. Vanin *et al. ibid* also suggests that his approach provides a means to generate high titre SIN vectors. However, there is no worked example and in fact no enabling disclosure because the skilled person would not have been aware, on the basis of the Vanin *et al.* paper, of the necessary modifications to make the suggested approach work. Vanin *et al.* makes no reference to hypoxic regulated vectors and/or regulated/inactivated lentiviral vectors.

15
20

LTRs

25 As already indicated, each retroviral genome comprises genes called *gag*, *pol* and *env* which code for virion proteins and enzymes. In the provirus, these genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for proviral integration, and transcription. They also serve as enhancer-promoter sequences. In other words, the LTRs can control the expression of the viral gene. Encapsidation of the retroviral RNAs occurs by virtue of a *psi* sequence located at the 5' end of the viral genome.

30

-14-

As used herein, the term "long terminal repeat (LTR) is used in reference to domains of base pairs located at the end of retroviral DNAs.

The LTRs themselves are identical sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

For ease of understanding, a simple, generic structures (not to scale) of the RNA and the DNA forms of the MLV retroviral genome is presented in Figure 7 in which the elementary features of the LTRs and the relative positioning of *gag/pol* and *env* are indicated. Please note that (i) *gag/pol* and *env* are normally not spaced apart; and (ii) the overlap normally present between the *pol* and *env* genes and the poly A tail normally present at the 3' end of the RNA transcript are not illustrated in Figure 7.

As shown in Figure 7, the basic molecular organisation of an infectious retroviral RNA genome is (5') R - U5 - *gag/pol*, *env* - U3-R (3'). In a defective retroviral vector genome *gag*, *pol* and *env* may be absent or not functional. The R regions at both ends of the RNA are repeated sequences. U5 and U3 represent unique sequences at the 5' and 3' ends of the RNA genome respectively.

Upon cellular transduction, reverse transcription of the virion RNA into double stranded DNA takes place in the cytoplasm and involves two jumps of the reverse transcriptase from the 5' terminus to the 3' terminus of the template molecule. The result of these jumps is a duplication of sequences located at the 5' and 3' ends of the virion RNA. These sequences then occur fused in tandem on both ends of the viral DNA, forming the long terminal repeats (LTRs) which comprise R U5 and U3 regions. On completion of the reverse transcription, the viral DNA is translocated into the nucleus where the linear copy of the retroviral genome, called a preintegration complex (PIC), is randomly inserted into chromosomal DNA with the aid of the virion integrase to form a stable provirus. The number of possible sites of integration into the host cellular genome is very large and very widely distributed.

-15-

Preferably the retroviral genome is introduced into packaging cell lines using retroviral transduction.

Preferably retroviral vector particles (such as MLV vector particles) are prepared in a transient expression system with a different envelope pseudotype to the packaging cell, and used to transduce a retroviral packaging cell.

Preferably the retroviral transduction step identifies retroviral insertions in integration sites that support high level expression of the resulting regulated retroviral genome.

10

Preferably stable transduced producer cell lines made by this initial retroviral transduction step produce retrovirus at titres of at least 10^6 per ml, such as from about 10^6 to about 10^7 per ml, more preferably at least about 10^7 per ml.

15 HIGH TITRE

As used herein, the term "high titre" means an effective amount of a retroviral vector or particle which is capable of transducing a target site such as a cell.

20 As used herein, the term "effective amount" means an amount of a regulated retroviral or lentiviral vector or vector particle which is sufficient to induce expression of an NOI at a target site.

Preferably the titre is from at least 10^6 retrovirus particles per ml, such as from about 10^6 to about 10^7 per ml, more preferably at least about 10^7 retrovirus particles per ml.

25

TRANSCRIPTIONAL CONTROL

The control of proviral transcription remains largely with the noncoding sequences of the viral LTR. The site of transcription initiation is at the boundary between U3 and R in the left hand side LTR (as shown in Figure 7) and the site of poly (A) addition (termination) is at the boundary between R and U5 in the right hand side LTR (as shown in Figure 7). The 3'U3 sequence contains most of the transcriptional control elements of the provirus,

30

-16-

which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins.

REGULABLE LTRs

5

AN LTR present, for example, in the construct of the invention and as a 3'LTR in the provirus of the producer cell of the invention may be a native LTR or a heterologous regulatable LTR. It may also be a transcriptionally quiescent LTR for use in SIN vector technology.

10

As used herein, the terms "regulatable LTR" and "regulatable 3'LTR" include vectors which contain responsive elements which are present in retroviral 3' LTR sequences, either by design or by their nature. As used herein, vectors comprising a "regulatable 3'LTR" are referred to as "regulated retroviral vectors". Within the regulatable 3'LTR region, the 3'U3 sequence contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins.

Responsive elements include but are not limited to elements which comprise, for example, promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins and/or elements which have been modified to render them inactive. As used herein, the term "modified" includes but is not limited to silencing, disabling, mutating, deleting or removing all of the U3 sequence or a part thereof.

25

The term "regulated LTR" also includes an inactive LTR such that the resulting provirus in the target cell can not produce a packagable viral genome (self-inactivating (SIN) vector technology) - see the Examples and Figure 6 for a particular embodiment.

30

ENHANCER

As used herein, the term "enhancer" includes a DNA sequence which binds other protein components of the transcription initiation complex and thus facilitates the initiation of transcription directed by its associated promoter.

In one preferred embodiment of the present invention, the enhancer is an ischaemic like response element (ILRE).

10 ILRE

The term "ischaemia like response element" - otherwise written as ILRE - includes an element that is responsive to or is active under conditions of ischaemia or conditions that are like ischaemia or are caused by ischaemia. By way of example, conditions that are like ischaemia or are caused by ischaemia include hypoxia and/or low glucose concentration(s).

The term "hypoxia" means a condition under which a particular organ or tissue receives an inadequate supply of oxygen.

20

Ischaemia can be an insufficient supply of blood to a specific organ or tissue. A consequence of decreased blood supply is an inadequate supply of oxygen to the organ or tissue (hypoxia). Prolonged hypoxia may result in injury to the affected organ or tissue.

25 A preferred ILRE is an hypoxia response element (HRE).

HRE

In one preferred aspect of the present invention, there is hypoxia or ischaemia regulatable expression of the retroviral vector components. In this regard, hypoxia is a powerful regulator of gene expression in a wide range of different cell types and acts by the induction of the activity of hypoxia-inducible transcription factors such as hypoxia inducible factor-1 (HIF-1; Wang & Semenza 1993 Proc Natl Acad Sci 90:430), which

30

-18-

bind to cognate DNA recognition sites, the hypoxia-responsive elements (HREs) on various gene promoters. Dachs *et al* (1997 Nature Med 5: 515) have used a multimeric form of the HRE from the mouse phosphoglycerate kinase-1 (PGK-1) gene (Firth *et al* 1994 Proc Natl Acad Sci 91:6496-6500) to control expression of both marker and
5 therapeutic genes by human fibrosarcoma cells in response to hypoxia *in vitro* and within solid tumours *in vivo* (Dachs *et al* *ibid*).

Hypoxia response enhancer elements (HREEs) have also been found in association with a number of genes including the erythropoietin (EPO) gene (Madan *et al* 1993 Proc Natl
10 Acad Sci 90: 3928; Semenza and Wang 1992 Mol Cell Biol 1992 12: 5447-5454). Other HREEs have been isolated from regulatory regions of both the muscle glycolytic enzyme pyruvate kinase (PKM) gene (Takenaka *et al* 1989 J Biol Chem 264: 2363-2367), the human muscle-specific β -enolase gene (ENO3; Peshavaria and Day 1991 Biochem J 275: 427-433) and the endothelin-1 (ET-1) gene (Inoue *et al* 1989 J Biol Chem 264: 14954-
15 14959).

Preferably the HRE of the present invention is selected from, for example, the erythropoietin HRE element (HREE1), muscle pyruvate kinase (PKM), HRE element, phosphoglycerate kinase (PGK) HRE, B-enolase (enolase 3; ENO3) HRE element,
20 endothelin-1 (ET-1)HRE element and metallothionein II (MTII) HRE element.

RESPONSIVE ELEMENT

Preferably the ILRE is used in combination with a transcriptional regulatory element ,
25 such as a promoter, which transcriptional regulatory element is preferably active in one or more selected cell type(s), preferably being only active in one cell type.

As outlined above, this combination aspect of the present invention is called a responsive element.

30

Preferably the responsive element comprises at least the ILRE as herein defined.

-19-

Non-limiting examples of such a responsive element are presented as OBHRE1 and XiaMac. Another non-limiting example includes the ILRE in use in conjunction with an MLV promoter and/or a tissue restricted ischaemic responsive promoter. These responsive elements are disclosed in WO99/15684.

5

Other examples of suitable tissue restricted promoters/enhancers are those which are highly active in tumour cells such as a promoter/enhancer from a *MUC1* gene, a *CEA* gene or a *5T4* antigen gene. The alpha fetoprotein (AFP) promoter is also a tumour-specific promoter. One preferred promoter-enhancer combination is a human
10 cytomegalovirus (hCMV) major immediate early (MIE) promoter/enhancer combination.

PROMOTER

The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase
15 binding site.

The promoter may be located in the retroviral 5' LTR to control the expression of a cDNA encoding an NOI.

20 Preferably the NOI is capable of being expressed from the retrovirus genome such as from endogenous retroviral promoters in the long terminal repeat (LTR)

Preferably the NOI is expressed from a heterologous promoter to which the heterologous gene or sequence is operably linked.

25

Alternatively, the promoter may be an internal promoter.

Preferably the NOI is expressed from an internal promoter.

30 Vectors containing internal promoters have also been widely used to express multiple genes. An internal promoter makes it possible to exploit promoter/enhancer combinations other than those found in the viral LTR for driving gene expression. Multiple internal promoters can be included in a retroviral vector and it has proved

-20-

possible to express at least three different cDNAs each from its own promoter (Overell *et al* 1988 Mol Cell Biol 8: 1803-1808). Internal ribosomal entry site (IRES) elements have also been used to allow translation of multiple coding regions from either a single mRNA or from fusion proteins that can then be expressed from an open reading frame.

5

TISSUE SPECIFIC PROMOTERS

The promoter of the present invention may be constitutively efficient, or may be tissue or temporally restricted in their activity.

10

Preferably the promoter is a constitutive promoter such as CMV.

Preferably the promoters of the present invention are tissue specific.

15 That is, they are capable of driving transcription of a NOI or NOI(s) in one tissue while remaining largely "silent" in other tissue types.

The term "tissue specific" means a promoter which is not restricted in activity to a single tissue type but which nevertheless shows selectivity in that they may be active in one
20 group of tissues and less active or silent in another group.

The level of expression of an NOI or NOIs under the control of a particular promoter may be modulated by manipulating the promoter region. For example, different domains within a promoter region may possess different gene regulatory activities. The roles of
25 these different regions are typically assessed using vector constructs having different variants of the promoter with specific regions deleted (that is, deletion analysis). This approach may be used to identify, for example, the smallest region capable of conferring tissue specificity or the smallest region conferring hypoxia sensitivity.

30 A number of tissue specific promoters, described above, may be particularly advantageous in practising the present invention. In most instances, these promoters may be isolated as convenient restriction digestion fragments suitable for cloning in a selected vector. Alternatively, promoter fragments may be isolated using the polymerase chain

-21-

reaction. Cloning of the amplified fragments may be facilitated by incorporating restriction sites at the 5' end of the primers.

5 The NOI or NOIs may be under the expression control of an expression regulatory element, such as a promoter and enhancer.

Preferably the ischaemic responsive promoter is a tissue restricted ischaemic responsive promoter.

10 Preferably the tissue restricted ischaemic responsive promoter is a macrophage specific promoter restricted by repression.

Preferably the tissue restricted ischaemic responsive promoter is an endothelium specific promoter.

15

Preferably the regulated retroviral vector of the present invention is an ILRE regulated retroviral vector.

20 Preferably the regulated retroviral vector of the present invention is an ILRE regulated lentiviral vector.

Preferably the regulated retroviral vector of the present invention is an autoregulated hypoxia responsive lentiviral vector.

25 Preferably the regulated retroviral vector of the present invention is regulated by glucose concentration.

30 For example, the glucose-regulated proteins (grp's) such as grp78 and grp94 are highly conserved proteins known to be induced by glucose deprivation (Attenello and Lee 1984 Science 226 187-190). The grp 78 gene is expressed at low levels in most normal healthy tissues under the influence of basal level promoter elements but has at least two critical "stress inducible regulatory elements" upstream of the TATA element (Attenello 1984 *ibid*; Gazit *et al* 1995 Cancer Res 55: 1660-1663). Attachment to a truncated 632 base

-22-

pair sequence of the 5' end of the *grp78* promoter confers high inducibility to glucose deprivation on reporter genes *in vitro* (Gazit *et al* 1995 *ibid*). Furthermore, this promoter sequence in retroviral vectors was capable of driving a high level expression of a reporter gene in tumour cells in murine fibrosarcomas, particularly in central relatively ischaemic/fibrotic sites (Gazit *et al* 1995 *ibid*).

Preferably the regulated retroviral vector of the present invention is a self-inactivating (SIN) vector.

By way of example, self-inactivating retroviral vectors have been constructed by deleting the transcriptional enhancers or the enhancers and promoter in the U3 region of the 3' LTR. After a round of vector reverse transcription and integration, these changes are copied into both the 5' and the 3' LTRs producing a transcriptionally inactive provirus (Yu *et al* 1986 Proc Natl Acad Sci 83: 3194-3198; Dougherty and Temin 1987 Proc Natl Acad Sci 84: 1197-1201; Hawley *et al* 1987 Proc Natl Acad Sci 84: 2406-2410; Yee *et al* 1987 Proc Natl Acad Sci 91: 9564-9568). However, any promoter(s) internal to the LTRs in such vectors will still be transcriptionally active. This strategy has been employed to eliminate effects of the enhancers and promoters in the viral LTRs on transcription from internally placed genes. Such effects include increased transcription (Jolly *et al* 1983 Nucleic Acids Res 11: 1855-1872) or suppression of transcription (Emerman and Temin 1984 Cell 39: 449-467). This strategy can also be used to eliminate downstream transcription from the 3' LTR into genomic DNA (Herman and Coffin 1987 Science 236: 845-848). This is of particular concern in human gene therapy where it is of critical importance to prevent the adventitious activation of an endogenous oncogene.

RETROVIRAL VECTORS

The regulated retroviral vector of the present invention includes but is not limited to: murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), feline immunodeficiency virus (FIV), caprine encephalitis-arthritis virus (CAEV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV),

-23-

Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV).

A detailed list of retroviruses may be found in Coffin *et al* ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

Preferred vectors for use in accordance with the present invention are retroviral vectors, such as MLV vectors.

Preferably the recombinant retroviral vectors of the present invention are lentiviral vectors, more preferably HIV or EIAV vectors.

LENTIVIRAL VECTORS

The lentiviruses can be divided into primate and non-primate groups. Examples of primate lentiviruses include but are not limited to: the human immunodeficiency virus (HIV), the causative agent of human auto-immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV). The non-primate lentiviral group includes the prototype "slow virus" visna/maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BIV).

A distinction between the lentivirus family and other types of retroviruses is that lentiviruses have the capability to infect both dividing and non-dividing cells (Lewis *et al* 1992 EMBO. J 11: 3053-3058; Lewis and Emerman 1994 J. Virol. 68: 510-516). In contrast, other retroviruses - such as MLV - are unable to infect non-dividing cells such as those that make up, for example, muscle, brain, lung and liver tissue.

Preferred vectors for use in accordance with the present invention are recombinant retroviral vectors, in particular recombinant lentiviral vectors, in particular minimal lentiviral vectors which are disclosed in WO 99/32646 and in WO98/17815.

VECTOR

As used herein, a "vector" denotes a tool that allows or facilitates the transfer of an entity from one environment to another. In accordance with the present invention, and by way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell. Optionally, once within the target cell, the vector may then serve to maintain the heterologous DNA within the cell or may act as a unit of DNA replication. Examples of vectors used in recombinant DNA techniques include plasmids, chromosomes, artificial chromosomes or viruses.

OPERABLY LINKED

The term "operably linked" denotes a relationship between a regulatory region (typically a promoter element, but may include an enhancer element) and the coding region of a gene, whereby the transcription of the coding region is under the control of the regulatory region.

DERIVABLE

The term "derivable" is used in its normal sense as meaning a nucleotide sequence such as an LTR or a part thereof which need not necessarily be obtained from an vector such as a retroviral vector but instead could be derived therefrom. By way of example, the sequence may be prepared synthetically or by use of recombinant DNA techniques.

VECTOR PARTICLES

In the present invention, several terms are used interchangeably. Thus, "virion", "virus", "viral particle", "retroviral particle", "retrovirus", and "vector particle" mean virus and virus-like particles that are capable of introducing a nucleic acid into a cell through a viral-like entry mechanism. Such vector particles can, under certain circumstances, mediate the transfer of NOIs into the cells they infect. A retrovirus is capable of reverse

-25-

transcribing its genetic material into DNA and incorporating this genetic material into a target cell's DNA upon transduction. Such cells are designated herein as "target cells".

A vector particle includes the following components: a retrovirus nucleic acid, which may contain one or more NOIs, a nucleocapsid encapsidating the nucleic acid, the nucleocapsid comprising nucleocapsid protein of a retrovirus, and a membrane surrounding the nucleocapsid.

NUCLEOCAPSID

10

The term "nucleocapsid" refers to at least the group specific viral core proteins (*gag*) and the viral polymerase (*pol*) of a retrovirus genome. These proteins encapsidate the retrovirus-packagable sequences and themselves are further surrounded by a membrane containing an envelope glycoprotein.

15

Preferably a high titre retroviral vector is produced using a codon optimised *gag* and a codon optimised *pol* or a codon optimised *env*.

CODON OPTIMISATION

20

As used herein, the terms "codon optimised" and "codon optimisation" refer to an improvement in codon usage. By way of example, alterations to the coding sequences for viral components may improve the sequences for codon usage in the mammalian cells or other cells which are to act as the producer cells for retroviral vector particle production. This is referred to as "codon optimisation". Many retroviruses, including HIV and other lentiviruses, use a large number of rare codons and by changing these to correspond to commonly used mammalian codons, increased expression of the packaging components in mammalian producer cells can be achieved. Codon usage tables are known in the art for mammalian cells, as well as for a variety of other organisms.

30

Preferably a high titre lentiviral vector is produced using a codon optimised *gag* and a codon optimised *pol* or a codon optimised *env*.

-26-

Preferably a high titre retroviral vector is produced using a modified and/or extended packaging signal.

PACKAGING SIGNAL

5

As used herein, the term "packaging signal" or "packaging sequence" refers to sequences located within the retroviral genome which are required for insertion of the viral RNA into the viral capsid or particle. Several retroviral vectors use the minimal packaging signal (also referred to as the psi sequence) needed for encapsidation of the viral genome.

10 By way of example, this minimal packaging signal encompasses bases 212 to 563 of the Mo-MLV genome (Mann et al 1983: Cell 33: 153).

As used herein, the term "extended packaging signal" or "extended packaging sequence" refers to the use of sequences around the psi sequence with further extension into the gag
15 gene. The inclusion of these additional packaging sequences may increase the efficiency of insertion of vector RNA into viral particles.

Preferably a high titre lentiviral vector is produced using a modified packaging signal.

20 Preferably the lentiviral construct is based on an EIAV vector genome where all the accessory genes are removed except Rev.

ACCESSORY GENES

25 As used herein, the term "accessory genes" refer to a variety of virally encoded accessory proteins capable of modulating various aspects of retroviral replication and infectivity. These proteins are discussed in Coffin et al (ibid) (Chapters 6 and 7). Examples of accessory proteins in lentiviral vectors include but are not limited to tat, rev, nef, vpr, vpu, vif, vpx. An example of a lentiviral vector useful in the present invention is one which
30 has all of the accessory genes removed except rev.

Preferably the production of lentiviral vector particles is increased by about 10 fold in the presence of EIAV Rev.

-27-

ENV

If the retroviral component includes an *env* nucleotide sequence, then all or part of that sequence can be optionally replaced with all or part of another *env* nucleotide sequence such as, by way of example, the amphotropic Env protein designated 4070A or the influenza haemagglutinin (HA) or the vesicular stomatitis virus G (VSV-G) protein. Replacement of the *env* gene with a heterologous *env* gene is an example of a technique or strategy called pseudotyping. Pseudotyping is not a new phenomenon and examples may be found in WO-A-98/05759, WO-A-98/05754, WO-A-97/17457, WO-A-96/09400, 10 WO-A-91/00047 and Mebatsion *et al* 1997 Cell 90, 841-847.

In one preferred aspect, the retroviral vector of the present invention has been pseudotyped. In this regard, pseudotyping can confer one or more advantages. For example, with the lentiviral vectors, the *env* gene product of the HIV based vectors would 15 restrict these vectors to infecting only cells that express a protein called CD4. But if the *env* gene in these vectors has been substituted with *env* sequences from other RNA viruses, then they may have a broader infectious spectrum (Verma and Somia 1997 Nature 389:239-242). By way of example, workers have pseudotyped an HIV based vector with the glycoprotein from VSV (Verma and Somia 1997 *ibid*).

20

In another alternative, the Env protein may be a modified Env protein such as a mutant or engineered Env protein. Modifications may be made or selected to introduce targeting ability or to reduce toxicity or for another purpose (Valsecia-Wittman *et al* 1996 J Virol 70: 2056-64; Nilson *et al* 1996 Gene Therapy 3: 280-6; Fielding *et al* 1998 Blood 9: 1802 25 and references cited therein).

TARGET CELL

As used herein the term "target cell" simply refers to a cell which the regulated retroviral 30 vector of the present invention, whether native or targeted, is capable of infecting or transducing.

-28-

The lentiviral vector particle according to the invention will be capable of transducing cells which are slowly-dividing, and which non-lentiviruses such as MLV would not be able to efficiently transduce. Slowly-dividing cells divide once in about every three to four days including certain tumour cells. Although tumours contain rapidly dividing
5 cells, some tumour cells especially those in the centre of the tumour, divide infrequently.

Alternatively the target cell may be a growth-arrested cell capable of undergoing cell division such as a cell in a central portion of a tumour mass or a stem cell such as a haematopoietic stem cell or a CD34-positive cell.

10

As a further alternative, the target cell may be a precursor of a differentiated cell such as a monocyte precursor, a CD33-positive cell, or a myeloid precursor.

As a further alternative, the target cell may be a differentiated cell such as a neuron,
15 astrocyte, glial cell, microglial cell, macrophage, monocyte, epithelial cell, endothelial cell, hepatocyte, spermatocyte, spermatid or spermatozoa.

Target cells may be transduced either *in vitro* after isolation from a human individual or may be transduced directly *in vivo*.

20 NOI

In accordance with the present invention, it is possible to manipulate the viral genome or the regulated retroviral vector nucleotide sequence, so that viral genes are replaced or supplemented with one or more NOIs which may be heterologous NOIs.

25

The term "heterologous" refers to a nucleic acid sequence or protein sequence linked to a nucleic acid or protein sequence which it is not naturally linked.

With the present invention, the term NOI (i.e. nucleotide sequence of interest) includes
30 any suitable nucleotide sequence, which need not necessarily be a complete naturally occurring DNA sequence. Thus, the DNA sequence can be, for example, a synthetic DNA sequence, a recombinant DNA sequence (i.e. prepared by use of recombinant DNA techniques), a cDNA sequence or a partial genomic DNA sequence, including

-29-

combinations thereof. The DNA sequence need not be a coding region. If it is a coding region, it need not be an entire coding region. In addition, the DNA sequence can be in a sense orientation or in an anti-sense orientation. Preferably, it is in a sense orientation. Preferably, the DNA is or comprises cDNA.

5

The NOI(s) may be any one or more of selection gene(s), marker gene(s) and therapeutic gene(s). As used herein, the term "selection gene" refers to the use of a NOI which encodes a selectable marker which may have an enzymatic activity that confers resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed.

10

SELECTABLE MARKERS

Many different selectable markers have been used successfully in retroviral vectors. These are reviewed in "Retroviruses" (1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 444) and include, but are not limited to, the bacterial neomycin (neo) and hygromycin phosphotransferase genes which confer resistance to G418 and hygromycin respectively; a mutant mouse dihydrofolate reductase gene which confers resistance to methotrexate; the bacterial *gpt* gene which allows cells to grow in medium containing mycophenolic acid, xanthine and aminopterin; the bacterial *hisD* gene which allows cells to grow in medium without histidine but containing histidinol; the multidrug resistance gene (*mdr*) which confers resistance to a variety of drugs; and the bacterial genes which confer resistance to puromycin or phleomycin. All of these markers are dominant selectable and allow chemical selection of most cells expressing these genes. Other selectable markers are not dominant in that their use must be in conjunction with a cell line that lacks the relevant enzyme activity. Examples of non-dominant selectable markers include the thymidine kinase (*tk*) gene which is used in conjunction with *tk* cell lines.

Particularly preferred markers are blasticidin and neomycin, optionally operably linked to a thymidine kinase coding sequence typically under the transcriptional control of a strong viral promoter such the SV40 promoter.

30

-30-

NOIs WITH THERAPEUTIC AND/OR DIAGNOSTIC APPLICATIONS

In accordance with the present invention, suitable NOI sequences include those that are of therapeutic and/or diagnostic application such as, but are not limited to: sequences
5 encoding cytokines, chemokines, hormones, antibodies, engineered immunoglobulin-like molecules, a single chain antibody, fusion proteins, enzymes, immune co-stimulatory molecules, immunomodulatory molecules, anti-sense RNA, a transdominant negative mutant of a target protein, a toxin, a conditional toxin, an antigen, a tumour suppressor protein and growth factors, membrane proteins, vasoactive proteins and peptides, anti-
10 viral proteins and ribozymes, and derivatives thereof (such as with an associated reporter group). When included, such coding sequences may be typically operatively linked to a suitable promoter, which may be a promoter driving expression of a ribozyme(s), or a different promoter or promoters, such as in one or more specific cell types.

15 NOIs FOR TREATING CANCER

Suitable NOIs for use in the invention in the treatment or prophylaxis of cancer include NOIs encoding proteins which: destroy the target cell (for example a ribosomal toxin), act
as: tumour suppressors (such as wild-type p53); activators of anti-tumour immune
20 mechanisms (such as cytokines, co-stimulatory molecules and immunoglobulins); inhibitors of angiogenesis; or which provide enhanced drug sensitivity (such as pro-drug activation enzymes); indirectly stimulate destruction of target cell by natural effector cells (for example, strong antigen to stimulate the immune system or convert a precursor substance to a toxic substance which destroys the target cell (for example a prodrug
25 activating enzyme).

PRO-DRUG ACTIVATING ENZYMES

Examples of prodrugs include but are not limited to etoposide phosphate (used with
30 alkaline phosphatase; 5-fluorocytosine (with cytosine deaminase); Doxorubin-N-p-hydroxyphenoxyacetamide (with Penicillin-V-Amidase); Para-N-bis (2-chloroethyl)aminobenzoyl glutamate (with Carboxypeptidase G2); Cephalosporin nitrogen mustard carbamates (with B-lactamase); SR4233 (with p450 reductase);

-31-

Ganciclovir (with HSV thymidine kinase); mustard pro-drugs with nitroreductase and cyclophosphamide or ifosfamide (with cytochrome p450).

NOIs FOR TREATING HEART DISEASE

5

Suitable NOIs for use in the treatment or prevention of ischaemic heart disease include NOIs encoding plasminogen activators. Suitable NOIs for the treatment or prevention of rheumatoid arthritis or cerebral malaria include genes encoding anti-inflammatory proteins, antibodies directed against tumour necrosis factor (TNF) alpha, and anti-
10 adhesion molecules (such as antibody molecules or receptors specific for adhesion molecules).

BYSTANDER EFFECT

15 The expression products encoded by the NOIs may be proteins which are secreted from the cell. Alternatively the NOI expression products are not secreted and are active within the cell. In either event, it is preferred for the NOI expression product to demonstrate a bystander effector or a distant bystander effect; that is the production of the expression product in one cell leading to the killing of additional, related cells, either neighbouring or
20 distant (e.g. metastatic), which possess a common phenotype. Encoded proteins could also destroy bystander tumour cells (for example with secreted antitumour antibody-ribosomal toxin fusion protein), indirectly stimulated destruction of bystander tumour cells (for example cytokines to stimulate the immune system or procoagulant proteins causing local vascular occlusion) or convert a precursor substance to a toxic substance
25 which destroys bystander tumour cells (eg an enzyme which activates a prodrug to a diffusible drug). Also, the delivery of NOI(s) encoding antisense transcripts or ribozymes which interfere with expression of cellular genes for tumour persistence (for example against aberrant *myc* transcripts in Burkitts lymphoma or against *bcr-abl* transcripts in chronic myeloid leukemia. The use of combinations of such NOIs is also envisaged.

30

CYTOKINES

The NOI or NOIs of the present invention may also comprise one or more cytokine-encoding NOIs. Suitable cytokines and growth factors include but are not limited to:

5 ApoE, Apo-SAA, BDNF, Cardiotrophin-1, EGF, ENA-78, Eotaxin, Eotaxin-2, Exodus-2, FGF-acidic, FGF-basic, fibroblast growth factor-10 (Marshall 1998 Nature Biotechnology 16: 129).FLT3 ligand (Kimura *et al* (1997), Fractalkine (CX3C), GDNF, G-CSF, GM-CSF, GF- β 1, insulin, IFN- γ , IGF-I, IGF-II, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (72 a.a.), IL-8 (77 a.a.), IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18 (IGIF), Inhibin α , Inhibin β , IP-10, keratinocyte growth factor-2 (KGF-2), KGF,

10 Leptin, LIF, Lymphotoxin, Mullerian inhibitory substance, monocyte colony inhibitory factor, monocyte attractant protein (Marshall 1998 *ibid*), M-CSF, MDC (67 a.a.), MDC (69 a.a.), MCP-1 (MCAF), MCP-2, MCP-3, MCP-4, MDC (67 a.a.), MDC (69 a.a.), MIG, MIP-1 α , MIP-1 β , MIP-3 α , MIP-3 β , MIP-4, myeloid progenitor inhibitor factor-1

15 (MPIF-1), NAP-2, Neurturin, Nerve growth factor, β -NGF, NT-3, NT-4, Oncostatin M, PDGF-AA, PDGF-AB, PDGF-BB, PF-4, RANTES, SDF1 α , SDF1 β , SCF, SCGF, stem cell factor (SCF), TARC, TGF- α , TGF- β , TGF- β 2, TGF- β 3, tumour necrosis factor (TNF), TNF- α , TNF- β , TNIL-1, TPO, VEGF, GCP-2, GRO/MGSA, GRO- β , GRO- γ , HCC1, 1-309.

20

The NOI or NOIs may be under the expression control of an expression regulatory element, such as a promoter and/or a promoter enhancer as known as "responsive elements" in the present invention.

25 VIRAL DELIVERY SYSTEMS

When the regulated retroviral vector particles are used to transfer NOIs into cells which they transduce, such vector particles also designated "viral delivery systems" or "retroviral delivery systems". Viral vectors, including retroviral vectors, have been used

30 to transfer NOIs efficiently by exploiting the viral transduction process. NOIs cloned into the retroviral genome can be delivered efficiently to cells susceptible to transduction by a retrovirus. Through other genetic manipulations, the replicative capacity of the retroviral

-33-

genome can be destroyed. The vectors introduce new genetic material into a cell but are unable to replicate.

The regulated retroviral vector of the present invention can be delivered by viral or non-viral techniques. Non-viral delivery systems include but are not limited to DNA transfection methods. Here, transfection includes a process using a non-viral vector to deliver a gene to a target mammalian cell.

Typical transfection methods include electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection, liposomes, immunoliposomes, lipofectin, cationic agent-mediated, cationic facial amphiphiles (CFAs) (Nature Biotechnology 1996 14; 556), multivalent cations such as spermine, cationic lipids or polylysine, 1, 2,-bis (oleoyloxy)-3-(trimethylammonio) propane (DOTAP)-cholesterol complexes (Wolff and Trubetskoy 1998 Nature Biotechnology 16: 421) and combinations thereof.

Viral delivery systems include but are not limited to adenovirus vector, an adeno-associated viral (AAV) vector, a herpes viral vector, a retroviral vector, a lentiviral vector, or a baculoviral vector. These viral delivery systems may be configured as a split-intron vector. A split intron vector is described in WO 99/15683.

Other examples of vectors include *ex vivo* delivery systems, which include but are not limited to DNA transfection methods such as electroporation, DNA biolistics, lipid-mediated transfection, compacted DNA-mediated transfection.

The vector may be a plasmid DNA vector. Alternatively, the vector may be a recombinant viral vector. Suitable recombinant viral vectors include adenovirus vectors, adeno-associated viral (AAV) vectors, Herpes-virus vectors, or retroviral vectors, lentiviral vectors or a combination of adenoviral and lentiviral vectors. In the case of viral vectors, gene delivery is mediated by viral infection of a target cell.

If the features of adenoviruses are combined with the genetic stability of retro/lentiviruses then essentially the adenovirus can be used to transduce target cells to become transient retroviral producer cells that could stably infect neighbouring cells.

5 PHARMACEUTICAL COMPOSITION

The present invention also provides a pharmaceutical composition for treating an individual by gene therapy, wherein the composition comprises a therapeutically effective amount of a regulated retroviral vector according to the present invention. The
10 pharmaceutical composition may be for human or animal usage. Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient.

The composition may optionally comprise a pharmaceutically acceptable carrier, diluent,
15 excipient or adjuvant. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s), and other carrier agents that may aid or increase
20 the viral entry into the target site (such as for example a lipid delivery system).

Where appropriate, the pharmaceutical compositions can be administered by any one or more of: minipumps, inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch,
25 orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the
30 form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

DISORDERS

The present invention is believed to have a wide therapeutic applicability - depending on *inter alia* the selection of the one or more NOIs.

5

For example, the present invention may be useful in the treatment of the disorders listed in WO-A-98/05635. For ease of reference, part of that list is now provided: cancer, inflammation or inflammatory disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia, anorexia, acute
10 infection, HIV infection, shock states, graft-versus-host reactions, autoimmune disease, reperfusion injury, meningitis, migraine and aspirin-dependent anti-thrombosis; tumour growth, invasion and spread, angiogenesis, metastases, malignant, ascites and malignant pleural effusion; cerebral ischaemia, ischaemic heart disease, osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease,
15 atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis; periodontitis, gingivitis; psoriasis, atopic dermatitis, chronic ulcers, epidermolysis bullosa; corneal ulceration, retinopathy and surgical wound healing; rhinitis, allergic conjunctivitis, eczema, anaphylaxis; restenosis, congestive heart failure, endometriosis, atherosclerosis or endosclerosis.

20

In addition, or in the alternative, the present invention may be useful in the treatment of disorders listed in WO-A-98/07859. For ease of reference, part of that list is now provided: cytokine and cell proliferation/differentiation activity; immunosuppressant or immunostimulant activity (e.g. for treating immune deficiency, including infection with
25 human immune deficiency virus; regulation of lymphocyte growth; treating cancer and many autoimmune diseases, and to prevent transplant rejection or induce tumour immunity); regulation of haematopoiesis, e.g. treatment of myeloid or lymphoid diseases; promoting growth of bone, cartilage, tendon, ligament and nerve tissue, e.g. for healing wounds, treatment of burns, ulcers and periodontal disease and neurodegeneration;
30 inhibition or activation of follicle-stimulating hormone (modulation of fertility); chemotactic/chemokinetic activity (e.g. for mobilising specific cell types to sites of injury or infection); haemostatic and thrombolytic activity (e.g. for treating haemophilia and stroke); antiinflammatory activity (for treating e.g. septic shock or Crohn's disease); as

-36-

antimicrobials; modulators of e.g. metabolism or behaviour; as analgesics; treating specific deficiency disorders; in treatment of e.g. psoriasis, in human or veterinary medicine.

- 5 In addition, or in the alternative, the present invention may be useful in the treatment of disorders listed in WO-A-98/09985. For ease of reference, part of that list is now provided: macrophage inhibitory and/or T cell inhibitory activity and thus, anti-inflammatory activity; anti-immune activity, i.e. inhibitory effects against a cellular and/or humoral immune response, including a response not associated with inflammation;
- 10 inhibit the ability of macrophages and T cells to adhere to extracellular matrix components and fibronectin, as well as up-regulated fas receptor expression in T cells; inhibit unwanted immune reaction and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases,
- 15 inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular inflammatory disorders, respiratory distress syndrome or other cardiopulmonary diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other
- 20 glandular diseases, glomerulonephritis or other renal and urologic diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal diseases, periodontal diseases or other dental diseases, orchitis or epididymo-orchitis, infertility, orchidial trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or
- 25 inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic neuritis, intraocular inflammation, e.g. retinitis or cystoid macular oedema, sympathetic ophthalmia, scleritis, retinitis pigmentosa, immune and inflammatory components of degenerative fundus disease, inflammatory components of ocular trauma, ocular inflammation caused by
- 30 infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g. following glaucoma filtration operation, immune and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders

-37-

where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other
5 degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy, chronic neuropathy, Guillain-Barre syndrome, Sydenham chora, myasthenia gravis, pseudo-tumour cerebri, Down's Syndrome,
10 Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side
15 effects of surgery, bone marrow transplantation or other transplantation complications and/or side effects, inflammatory and/or immune complications and side effects of gene therapy, e.g. due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases, e.g. leukaemia, by reducing the amount of
20 monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

INTRODUCTION TO THE EXAMPLES SECTION AND THE FIGURES

25

The present invention will now be described only by way of example in which reference is made to the following Figures:

Figure 1 shows an MLV-based transduction method using a Cre/LoxP system as
30 described by Vanin *et al* *ibid* (1997);

Figure 2 shows an EIAV-based transduction method using a Cre/Lox system;

-38-

Figure 3 shows an MLV SIN vector construct transduction method with an EIAV/HIV genome insertion using a Cre/Lox system;

5 Figure 4 shows an MLV-based transduction method with HRE 3'LTR using a Cre/Lox P system;

Figure 5 shows an MLV-based transduction method for MLV SIN vector production using a Cre/Lox P system;

10 Figure 6 shows an MLV-based transduction method with integration of a complete second genome construct using a Cre/LoxP system;

Figure 7 shows the basis molecular organisation of an RNA genome and a proviral DNA genome;

15

Figure 8 shows a schematic diagram of pTrap2 and pONY8z-loxP plasmids;

Figure 9 shows an overall summary of the recombinase method;

20 Figure 10a shows a FACS analysis of EV1 packaging cells prior to transduction with Trap2 vector;

Figure 10b shows FACS analysis of EV1 packaging cell line transduced with Trap2 at an MOI of 0.3. A 5% top slice of the highest expressers was carried out;

25

Figure 11 shows a validation of the method for quantitation of GFP mRNA, relative to β -actin. A titration of the total RNA from EV1 clone A was used. The difference in Ct values between the two assays is shown on the y axis. The magnitude of the gradient must be <0.1 for the method to be valid. The gradient is 0.077, so the method is suitable;

30

Figure 12 shows the quantitation of GFP mRNA relative to control β -actin mRNA. EV2 TD cells are transduced with Trap2 at an MOI of 0.3 and are the calibrator sample with the ratio designated 1.0;

-39-

Figure 13 shows FACS analysis of EV1 clone A:

Figure 13A shows original GFP expression of the clone;

- 5 Figure 13B shows GFP expression 7 days after transfection with Cre recombinase (pBS185). Excision frequency is 64%;

Figure 13C shows recombined clone 4 identified as being negative for GFP:

- 10 Figure 14 shows lacZ expression of transfected cells with and without the addition of the Cre recombinase (pBS185). Figure 14 shows EV1A4 and EV2D4 clones with and without the addition of Cre recombinase (pBS185). The efficiency of the insertion event was estimated to be about 12% by computer image analysis;

- 15 Figure 15 shows the structure of pONY8.1Z MLVHyb;

- Figure 16 shows the alignment of leader and gag regions present in vectors pONY4Z, 8Z and ATG mutated 8Z vector. The latter is referred to as pONY8ZA. The sequences aligned are from the NarI site in the leader to the XbaI site between the EIAV gag sequence and the CMV promoter. Sequences in the leader are shown in italic and a space is present upstream of the position of the gag ATG; and
- 20

Figure 17 shows a schematic representation of the structure of pONY 8.3G +/- vector genome plasmids.

25

EXAMPLES

EXAMPLE 1

- 30 Vanin *et al* (*ibid*) describe a recombinase system whereby an initial retroviral transduction event introduces retroviral LTRs and expressed gene/s flanked by two recombinase target sites (exemplified by loxP) into a cell line. Stable transduced cell lines are selected by resistance to the antibiotic neomycin and screened for high expression of

-40-

the expressed gene(s) (see Figure 1). Such cell lines (Cell Line 1) contain retroviral insertions in integration sites that support high level expression from the retroviral genome.

- 5 The next step involves the transfection of the relevant recombinase expression construct (exemplified here by Cre recombinase) into the identified high expressing cell line. The expressed gene(s) is/are excised and a single loxP site is retained in the construct (Cell Line 2). In this instance, thymidine kinase gene (tk) is used as a negative selectable marker in combination with the drug, gancyclovir. The final step involves the re-
- 10 insertion of a therapeutic or marker gene of choice into the single loxP site via a Cre-assisted mechanism. Cell lines are identified that have been successfully recombined (Cell Line 3) and they will produce retroviruses at the same titre as the parental Cell Line 1.

15 EXAMPLE 2

Figure 2 and Figure 3 describe the production of EIAV or HIV high titre transduced producer cell lines.

- 20 Figure 2 shows a minimal EIAV genome construct with the 3' U3 sequences replaced by a strong constitutive promoter, CMV. A reporter gene such as blasticidin resistance gene (*bsr*) is flanked by loxP sites. Virus is made in a transient system and is transduced into an EIAV producer cell line and clones identified that maximally express the blast marker gene. A line is chosen (termed Cell Line 1) and the marker gene is excised by a Cre
- 25 recombinase-assisted excision event, generating Cell Line 2.

- Construct B comprises two loxP sites which flank an internal expression cassette and also the native EIAV 3' LTR. Therefore, this construct is recombined into the cell line such that the 5' R and U5 sequences are inherited from the packaging cell line, whereas the 3'
- 30 LTR sequences are wholly derived from the recombined construct. The 3' LTR from Cell Line 2 is present downstream of the functional EIAV genome expression construct. This CMV-R-U5 module is still transcriptionally active but expression is directed away from the EIAV genome.

Figure 3 shows a further aspect of the invention. Construct C is based on an MLV SIN vector, with a deletion in the 3' U3 sequences. The cassette includes an internal CMV promoter linked to EIAV R and U5 sequences. This is followed by a blasticidin resistance gene (*bsr*) flanked by two loxP sites. Virus is made in a transient transfection system and the genome is transduced into a packaging line. Blast-resistant clones are identified and the highest expressing line is chosen for further analysis. This line is transfected with Cre recombinase and the blast gene is excised. The last step involves the insertion of construct B into the single loxP site. Once again, a complete EIAV 3' LTR is introduced into the producer cell line. This leads to a CMV-driven EIAV genome expression cassette with the EIAV 3' LTR still located at the 3' end of the genome. Transcriptionally quiescent MLV SIN LTRs flanks these EIAV sequences.

EXAMPLE 3

Figure 4 shows an additional aspect of the invention. Construct D is an MLV-based vector with a CMV promoter in the 3' LTR in place of the U3 sequences. Virus is made in a transient system and is transduced into a packaging cell line as described previously. The neo and TK genes are excised by the action of Cre recombinase and construct E is recombined into the single loxP target sequence. The modified MLV 3' LTR including the HRE or similarly regulated system is transferred into the packaging cell line by the recombinase mechanism. Therefore, the 5' R and U5 sequences are inherited from the producer cell line whereas the therapeutic and marker gene/s and regulated 3' LTR is inherited from construct E. The final producer cell line is constitutively driven by the 5' CMV promoter and will produce high titre retroviral vectors which are regulated in the transduced target cells. This approach avoids the derivation of low titre transfected producer cell lines or the use of hypoxic conditions or chemical mimics for production from traditionally derived transduced producer lines.

EXAMPLE 4

Figure 5 shows yet another aspect of the invention. Construct D is an MLV-based vector with a CMV promoter in the 3' LTR as previously described. The same process is carried

-42-

out as shown in Figure 4 until the final recombination is performed. Construct F contains a deletion in U3 sequences in the 3' LTR and an internal expression cassette comprising a promoter and gene sequences. The final cell line containing the Cre-mediated recombination will be CMV-driven and will constitutively produce high titre MLV SIN
5 vectors. Previously, SIN vectors have not been amenable to production by stable cell line producer technology. Instead they have been prepared using transfection-based transient expression systems.

EXAMPLE 5

10

Figure 6 shows an MLV-based transduction method with integration of complete second genome construct by Cre/LoxP system. In this approach, construct 1 (called TRAP1) is an MLV vector construct containing an internal CMV promoter operably linked to a marker gene (a truncated form of the human low affinity nerve growth factor receptor,
15 called LNGFR). The enhancer elements in the 3' U3 sequence have been excised and replaced by a 34bp loxP site. Virus stocks are prepared in a transient system and the TRAP1 genome is stably transduced into packaging cell lines.

The modified 3'U3 sequences, including the lox P sequence, is copied from the 3'LTR
20 position to the 5'LTR, such that there is little 5' promoter activity. Cell lines are screened for high levels of expression of LNGFR protein by fluorescent activated cell sorter (FACS) analysis and clonal lines are derived by standard techniques. A Cre recombinase expression plasmid is transfected into the derived cell line to excise all sequences between the two loxP sites. Next, cells are negatively selected by FACS for absence of
25 LNGFR expression and clonal lines are derived by standard techniques. Construct 2 in this example comprises a complete HIV or EIAV or also MLV retroviral genome, which is flanked by two minimal 34bp loxP recombinase sites. A strong constitutive promoter such as CMV directs transcription of the genome. On transfection of plasmid 2 and Cre expression plasmid, the complete lentivirus vector or MLV vector genome is inserted in
30 the producer cell line. These sequences are flanked to the 5' by a small portion of MLV U3 sequence and a loxP site and to the 3' by the second loxP site, enhancerless-U3 sequences, R and U5 derived from the MLV construct 1.

Derivation of Plasmid TRAP1 (Figure 6 - Construct 1)

Oligonucleotides VSAT129 and VSAT130 were synthesised which correspond to the
5 minimal 34bp loxP sites and contain a 5' overhang for NheI and a 3' overhang for XbaI.
The sequences 5' to 3' are as follows: VSAT129 (CTAGCATAACTTCGTATA
ATGT.ATGCTATACGAAGTTATT) (SEQ ID No 49) and VSAT130
(CTAG.AATAACTTCGTATAGC ATACATTATACGAAGTTATG) (SEQ ID No 50).
The two oligonucleotides were treated with T4 polynucleotide kinase and were heated to
10 95°C for 5 minutes, before gradual cooling to room temperature. The annealed and
kinased oligos were ligated to a 2,830 bp NheI/XbaI fragment from LTR plasmid (SEQ
ID No 59). Fragments were ligated and correct clones of LTRloxP were identified by
sequence analysis. Plasmid LTRloxP was then digested with NheI and ScaI and a
2.185bp fragment was prepared for following cloning steps.

15

Plasmids TRAP1 and TRAP1G were derived from LTRloxP and the MLV genome
CGCLNGFR (encodes GFP and LNGFR from an internal CMV promoter – see SEQ ID
No 57). However, the GFP gene was excised by EcoRI/BsmI digestion and the 6,796bp
fragment was filled in by T4 DNA polymerase and re-ligated, in order to generate
20 plasmid CXCLNGFR. Plasmid TRAP1 was generated by ligation of a 2,185bp NheI/ScaI
fragment from LTRloxP (see SEQ ID No 58) to a 4,426bp NheI/ScaI fragment from
CXCLNGFR. Plasmid TRAP1G was generated by ligation of a 2,185bp NheI/ScaI
fragment from LTRloxP to a 5,179bp NheI/ScaI fragment from CGCLNGFR.

25 Derivation of Plasmid pONY8z-lox (Figure 6 - Construct 2)

In this example, the retroviral genome inserted into the loxP site in Figure 6 was based on
the EIAV vector genome, pONY8z (for preparation see pONY8.0Z construction below).
pONY8z was cut with SnaBI and NruI, and the 4358bp fragment purified and self-ligated
30 to form pONY8z-shuttle. This plasmid has unique 5' sites (DraIII and BglII) and unique
3' sites (PvuII and BspLUII). Oligonucleotides encoding the 34bp loxP sites were
inserted with suitable base pair overhangs at the unique 5' DraIII site and then the unique
3' BspLUII, to generate plasmid pONY-8z-shuttleloxP.

Plasmid pONY8z-loxP was made as follows. Plasmid pONY-8z-shuttleloxP was digested with BsrG I and NspV, and the 3670bp fragment was purified as the vector fragment. The insert for ligation to this fragment was derived from pONY8z by partial
5 digestion with BsrGI (two sites) followed by digestion with NspV. A 7,328bp fragment was purified and ligated to the 3670bp fragment described above.

The Cre recombinase plasmid as used in this system is pBS185 (Gibco).

10 EXAMPLE 6

We constructed an MLV self-inactivating (SIN) vector called pTrap2 (see SEQ ID No 56) by replacing the 3' U3 NheI-XbaI fragment with a 34-bp loxP sequence. The vector transcribes the marker gene GFP from an internal CMV promoter. Trap2 vector was used
15 to transduce EIAV packaging cell lines EV1 and EV2. The EV cell lines are based on human TE671 cells and express EIAV gag/pol proteins and VSV-G envelope, regulated by a temperature-sensitive switch. High expressing clones of transduced EV1 and EV2 cells were identified by FACS analysis for GFP. Individual clones expressing high levels of GFP were then selected. The GFP expression cassette was excised following transient
20 transfection with a Cre recombinase expression plasmid. The derived cell line, EV-loxP, contains a single loxP site and minimal sequences derived from the MLV construct pTrap2. An EIAV genome was engineered to contain loxP sites flanking the entire vector genome.

25 This genome construct and Cre recombinase were co-transfected into EV-loxP. Stable cell lines expressing lacZ were selected by FACS and cell lines were cloned by limiting dilution. Therefore, we have introduced an entire EIAV genome expression cassette into a single loxP site. This site was previously identified by MLV transduction as highly permissive for transgene expression. A 5' CMV promoter transcribes the lentiviral
30 genome in the producer cell line but the expression site was originally identified by MLV transduction. This method is adaptable to the generation of transduced producer cell lines for other lentiviral vector systems.

Materials and Methods

Vector construction: Plasmid pTrap2 was made as follows: A plasmid containing a single MLV LTR plasmid (LTRplasmid – SEQ ID No 59) was digested with *NheI* and *XbaI* and a 34 bp minimal loxP site was introduced with relevant sticky ends. This insertion step removes the MLV U3 enhancer elements which lie within the excised *NheI*-*XbaI* fragment. The LTR-loxP plasmid was linearised by digestion with *NheI* and was ligated to a 6.8kb *NheI* fragment from the MLV construct CZCG (See SEQ ID No 55). This construct expresses lacZ from the 5' U3 promoter and GFP from an internal CMV promoter. The resulting pTrap2 construct is shown in Figure 8.

The EIAV genome construct pONY8.0Z and pONY8.1Z were prepared as follows:

pONY8.0Z construction

pONY8.0Z was derived from pONY4.0Z (see WO 99/32646) by introducing mutations which 1) prevented expression of TAT by an 83nt deletion in the exon 2 of tat) prevented S2 ORF expression by a 51nt deletion 3) prevented REV expression by deletion of a single base within exon 1 of rev and 4) prevented expression of the N-terminal portion of gag by insertion of T in ATG start codons, thereby changing the sequence to ATTG from ATG. With respect to the wild type EIAV sequence Acc. No. U01866 these correspond to deletion of nt 5234-5316 inclusive, nt 5346-5396 inclusive and nt 5538. The insertion of T residues was after nt 526 and 543.

pONY8.1Z construction

pONY8.1Z was obtained directly from pONY8.0Z by digestion with *Sall* and partial digestion with *SapI*. Following restriction the overhanging termini of the DNA were made blunt ended by treatment with T4 DNA polymerase. The resulting DNA was then religated. This manipulation results in a deletion of sequence between the LacZ reporter gene and just upstream of the 3'PPT. The 3' border of the deletion is nt 7895 with respect to wild type EIAV, Acc. No. U01866. Thus pONY8.1Z does not contain sequences corresponding to the EIAV RREs.

Plasmid pONY8z was linearised by *Bgl*II, and a single loxP site was cloned into *Bgl*II, immediately upstream of the 5' CMV promoter, to produce pONY8z-loxP. Plasmids pONY3.2iresHYG and pHCMV-VSVG were used in the derivation of cell lines EV1 and EV2. The plasmid pONY3.2iresHYG was constructed as follows:

pONY3.2IRES_{hyg}

pONY3.IRES_{hyg} was derived from pONY3.2. pONY3.2 is a derivative of pONY3.1 in which expression of TAT and S2 are ablated by an 83nt deletion in the exon 2 of tat a 51nt deletion in S2 ORF. With respect to the wild type EIAV sequence Acc. No. U01866 these correspond to deletion of nt 5234-5316 inclusive and nt 5346-5396 inclusive. This fragment was introduced into the expression vector pHORSE IRES _{hyg} which was made as follows. pHORSE (see WO 99/32646) was cut with *Sna*BI and *Not*I which excises a fragment running from the CMV promoter through EIAV gag/pol and introduced into pIRES1_{hyg} (Clontech) digested with the same enzymes. This plasmid was then cut with *Sse*8387I and *Bst*EII and then ligated with the *Sse*8387I to *Bst*EII fragment from pONY3.2. The sequence of the plasmid is set out in SEQ ID No 51.

20 Virus Production

Transient MLV vector preparations pseudotyped with RD114 cat endogenous envelope were made as described previously (Soneoka et al., 1995). EIAV vector was harvested from confluent monolayers following 3 days induction of VSV-G expression at 32°C. MLV vector preparations were titred in triplicate on HT1080 fibrosarcoma cells. EIAV vector preparations were titred by GFP and lacZ on D17 dog osteosarcoma cells.

Flow cytometry of b-galactosidase and GFP activity:

30 1.5×10^5 cells from a 12-well plate were analysed for lacZ expression using the FluoReporter lacZ Flow Cytometry kit (Molecular Probes). GFP expression was also directly assessed using the FACSCalibur flow cytometer (Beckton Dickinson).

Transfection methods

Calcium phosphate transfections were carried out using the Profection kit (Promega) according to manufacturer's instructions.

5 Results

Figure 8 shows a schematic diagram of pTrap2 and pONY8z-loxP, plasmids used in this study.

10 Introduction of Trap2 genome into EV1 and EV2

An overall summary of the process described here is given in Figure 9. Trap2 MLV vector was made in a transient system with the amphotropic 4070A envelope. It gave a GFP titre of 1.7×10^6 T.U. per ml. Trap2 vector however also gave a lacZ titre of 9.4×10^5 T.U. per ml. This shows that replacement of the *NheI-XbaI* fragment from the MLV U3 region with loxP does not completely inactivate the MLV U3 promoter. Therefore Trap2, as constructed, is a partial SIN vector.

EV1 and EV2 cells were transduced with Trap2 vector at a multiplicity of infection (MOI) of 0.3. This was done to insert single copies of the MLV genome into the packaging lines.

Derivation of high expressers of GFP marker gene

25 Transduced EV1 and EV2 cells were analysed by FACS (see Figure 10) and the top 5% of GFP expressing cells were sorted and expanded. Clonal lines were derived by limiting dilution and four clones of EV1 and EV2 were chosen by visual inspection.

A quantitative TaqMan RT-PCR reaction was established in order to identify which of the four clones of EV1 and EV2 were the highest expressors of GFP mRNA. Total RNA was analysed by RT-PCR for GFP and β -actin. Quantitation was calculated by direct comparison of the Ct values (Cycle threshold). This was possible as it was proved that the two individual RT-PCR reactions are of similar efficiency (see Figure 11). By identifying an

-48-

optimal chromosomal location for GFP transgene expression, we can ensure that the inserted loxP site will be highly permissive for expression of an inserted lentiviral genome construct.

5 Figure 5 shows the n-fold difference in GFP : β -actin ratio for clones EV1 A to D and EV2 A to D. All ratios are defined relative to a calibrator sample, defined as a ratio of 1.0. The calibrator sample used was RNA from EV2 cells transduced with Trap2 at an MOI of 0.3.

10 This identified the best expressing lines as:

- EV1 clone A - GFP : β -actin ratio is 22.8
- EV2 clone D - GFP : β -actin ratio is 18.6

These two lines were carried forward for further study.

15

Excision of internal expression cassette by Cre recombinase

The process of retroviral integration copies the loxP-containing modified 3' U3 to the 5' position. Therefore, one can excise the majority of the MLV Trap2 integration by the
20 action of Cre recombinase. This will leave a single modified LTR, suitable for lentiviral genome integration.

EV1 clone A and EV2 clone D were transfected by the Cre expression plasmid pBS185 (Life Technologies). After one week, the cells were analysed for GFP by FACS (see
25 Figure 13) to determine the excision frequency. This was measured at 20-70% in all lines.

Recombined clones were identified by limit dilute cloning cells and checking by microscope and FACS for loss of GFP expression.

30

Insertion of EIAV genome into loxP site

-49-

Plasmid pONY8x-loxP and pBS185 were co-transfected using Fugene into EV1 clone A (excised) and EV2 clone D (excised). A control transfection of pONY8z-loxP in the absence of pBS185 was also carried out.

- 5 Figure 14 shows lacZ expression of transfected cells with and without the addition of Cre recombinase (pBS185). The efficiency of the insertion event was estimated to be ~12% by computer image analysis.

- 10 We analysed cells for lacZ expression by FACS using the FluoReporter lacZ Flow Cytometry kit. The top 5% of lacZ positive cells were sorted by FACS and clones were derived by limiting dilution. In total, 12 clones of EV1/A/pONY8z-loxP were derived and 13 clones of EV2/D/pONY8z-loxP.

15 EXAMPLE 7

Construction of EIAV vectors with LTR driven open reading frames

- 20 The EIAV vector configurations described previously utilise a single promoter - transgene cassette located internally in the vector. For example in pONY8Z the promoter-transgene cassette is CMV-LacZ. However for some uses it would be advantageous to have the option of expressing a gene from the 5'LTR promoter as well. For example a marker gene such as green fluorescent protein (GFP), a resistance marker such as neomycin phosphotransferase (neo) or another protein or a biologically active
- 25 entity such as a ribozyme. Previous experiments have shown that the EIAV LTR is weakly active in human cells in the absence of EIAV tat. However the transcriptional activity of the LTR can be increased by replacement of the EIAV U3 region with the MLV U3 region or the CMV promoter. This is achieved by introducing these alterations in the 3'LTRs of the vector plasmids. As a result of the replicative strategy of
- 30 retroviruses the modified 3'LTR becomes positioned at the 5'end of the integrated vector and can thus drive expression of a gene placed downstream of the gag region. To ensure optimal levels of expression there should preferably be no ATG start codons prior to the start codon of the gene to be expressed. In pONY8Z the ATG start codon of gag and the next ATG downstream were mutated to ATTG in order to ablate expression of the

-50-

aminoterminal portion of gag present in the vectors, however there are 7 other ATG codons further downstream of these, within gag, from which translation might be initiated.

- 5 Described below are the replacement of the U3 region of EIAV with MLV or CMV promoters and the mutation of ATG codons in the gag region

Replacement of the EIAV U3 region with MLV U3 or CMV promoters

- 10 The MLV U3 region was introduced into pONY8Z vector by replacement of the 3'LTR with a synthetic MLV/EIAV LTR made by the overlapping PCR technique, using the following primers and templates.

The EIAV PPT/U3 sequence was amplified from pONY8.1Z using primers:

- 15 KM001: CAAAGCATGCCTGCAGGAATTCG (SEQ ID No 1)

and

KM003:

- 20 GCCAAACCTACAGGTGGGGTCTTTCATTATAAAACCCCTCATAAAAACCCAC
AG (SEQ ID No 2)

to give the following product:

- 25 CAAAGCATGCCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGAATTG
GAAGAGCTTTAAATCCTGGCACATCTCATGTATCAATGCCTCAGTATGTTTAG
AAAAACAAGGGGGGAAGTGTGGGGTTTTATGAGGGGTTTTATAATGAAAGA
CCCCACCTGTAGGTTTGGC (SEQ ID No 3)

- 30 The MLV U3 region was amplified from pHIT111 (Soneoka et al., (1995) Nucleic Acids Res. 23, 628-633) using KM004:

-51-

CTGTGGGGTTTTTATGAGGGGTTTTATAATGAAAGACCCACCTGTAGGTTTG

GC (SEQ ID No 4)

and

5 KM005:

GAAGGGACTCAGACCGCAGAATCTGAGTGCCCCCGAGTGAGGGGTTGTGGG

CTCT (SEQ ID No 5) to give the following product:

10 CTGTGGGGTTTTTATGAGGGGTTTTATAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCT
TAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATC
AAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTT
CCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCT
GTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGC
15 CCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCC
TGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTGCGCGCTTCTGCTCCCCG
AGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGGGCACTCAGATTCTGCGGTCTGAGTCC
CTTC (SEQ ID No 6)

20 The MLV U3/EIAV R/U5 was amplified from pONY8.1Z using primers

KM002: GAGCGCAGCGAGTCAGTGAGCGAG (SEQ ID No 7) and

KM006:

25

AGAGCCCACAACCCCTCACTCGGGGGGCACTCAGATTCTGCGGTCTGAGTCC
CTTC (SEQ ID No 8)

to give the following product:

30

AGAGCCCACAACCCCTCACTCGGGGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTG
GGCTGAAAAGGCCTTTGTAATAAATATAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTT
CGAGATCCTACAGAGCTCATGCCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTG
TTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGC
35 CTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCGCTTTCAGTCGGGAAAC

-52-

CTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGG
CGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTC (SEQ ID No 9)

The PCR products described above were purified and then used as templates in new PCR reactions to link them together to obtain a 992bp product. The final product contains two SapI sites which flank the hybrid LTR. These allow introduction of the PCR product into the corresponding SapI sites present in the pONY8Z or pONY8.1Z vector plasmid, thereby creating pONY8Z MLVHyb and pONY8.1 MLVHyb. The sequence of the hybrid LTR in these plasmids was confirmed by sequencing. The titres obtained from the vectors in transient transfection assays are shown in Table 1. The titres were very similar to the titres from the parental construct pONY8Z and pONY8.1Z indicating that replacement of the EIAV U3 region with that of MLV had little or no detrimental effect on the infectious cycle of the vectors.

Table 1. Titres obtained from MLV hybrid LTR vector plasmids

vector plasmid	[#] titre (l.f.u./ml)
pONY8Z	3 x 10 ⁵
pONY8Z MLVHyb	1 x 10 ⁵
pONY8.1Z	6 x 10 ⁴
pONY8.1Z MLVHyb	2 x 10 ⁴

[#] Titre was measured on D17 cells and is expressed as LacZ forming units/ml (l.f.u./ml). Transfections were carried out in 293T cells using the vector plasmid shown and pRV67 (VSV-G expression plasmid), and pONY3.1 (EIAV gag/pol expression plasmid).

The structure of pONY8.1Z MLVHyb is shown in Figure 15 and the sequence of this plasmid is shown as SEQ ID No 10.

The EIAV promoter was also replaced by the human cytomegalovirus (CMV) promoter using a similar strategy. The primers and templates were the same except that KM003 was replaced by KM008:

-53-

GGCCATCGTGCCTCCCCACTCCTGCAGTTATAAAACCCCTCATAAAACCCCA
CAG (SEQ ID No 11)

KM004 was replaced by KM009:

5

CTGTGGGGTTTTTATGAGGGGTTTATAAACTGCAGGAGTGGGGAGGCACGA
TGGCC (SEQ ID No 12)

KM005 was replaced by KM010:

10

GAAGGGACTCAGACCGCAGAATCTGAGTGCCCGGTTCACTAAACGAGCTCTG
CTTATATAGACC (SEQ ID No 13) and

KM006 was replaced by KM011:

15

GGTCTATATAAGCAGAGCTCGTTTAGTGAACCGGGCACTCAGATTCTGCG
GTCTGAGTCCCTTC (SEQ ID No 14)

The template for the PCR reaction with primers KM009 and KM010 was pONY2.1LacZ.

20 This plasmid contains a single CMV promoter. The combined PCR product of 1319 bp was digested with SspI and introduced into the pONY8Z or pONY8.1Z backbone as described above for pONY8Z MLVHyb.

Mutation of remaining ATG codons in the gag of pONY8Z to ATTG

25

The alignment of the sequence of the leader and gag region present in vectors pONY4Z (an earlier generation ELAV vector), pONY8Z and a derivative of pONY8Z in which the 7 remaining ATG codons are mutated to ATTG is shown in Figure 16. These mutations were created by PCR mutagenesis as follows. The template for the PCR reactions was
30 pONY8Z and the primers were:

F1: CGAGATCCTACAGTTGGCGCCCGAACAG (SEQ ID No 15);

-54-

R1:GAGTTACAATCTTCCAGCAATGGAATGACAATCCCTCAGCTGCCAGTCCTT
TTCTTTTACAAAGTTGGTATCAATGAAATAAGTCTACTAGACTTAGC (SEQ ID
No 16);

5 F2:TTCCATTGCTGGAAGATTGTAACCTCAGACGCTGTCAGGACAAGAAAGAGA
GGCCTTTGAAAGAACATTGGTGGGCAATTTCTGCTGTAAAGATTG (SEQ ID No
17);

R2:CAATATTTTCGCTCTTAGGAGCTGGAATGATGCCTTTCCAATCTACTACAAT
10 TATTAATCTGGAGGCCCAATCTTTACAGCAGAAATTGCCACCAATG (SEQ ID
No 18);

R3:CCACTAGTTCTAGAGATATTCTTCAGAGGGCTCAGACTGCTTTTTATTAGC
AGTCTTCTTTTCAATATTTTCGCTCTTAGGAGC (SEQ ID No 19)

15

In the first stage of construction two PCR reactions were set up with primer pairs F1/R1
and F2/R2, respectively. These were purified and then used in a second 'overlapping'
reaction in which primers F1 and R3 were added after 10 cycles. This procedure results
in a 552bp PCR product (SEQ ID No 20):

20

CGAGATCCTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAA
CCTGGCTGATCGTAGGATCCCCGGGACAGCAGAGGAGAACTTACAGAAGTCTTCTGGAGGTGT
TCCTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCTTTGACATTGGAGCAAGGCG
CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAATACTGGTAACTGTAAT
25 TGGGCGCTAAGTCTAGTAGACTTATTTCAATGATACCAACTTTGTAAAAGAAAAGGACTGGCA
GCTGAGGGATTGTCATTCCATTGCTGGAAGATTGTAACCTCAGACGCTGTCAGGACAAGAAAGA
GAGGCCTTTGAAAGAACATTGGTGGGCAATTTCTGCTGTAAAGATTGGGCCTCCAGATTAATA
ATTGTAGTAGATTGGAAAGGCATCATTCCAGCTCCTAAGAGCGAAATATTGAAAAGAAGACTG
CTAATAAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAACTAGTGG

30

This was digested with *NarI* and *XbaI* and ligated into pONY8Z. pONY8Z MLVHyb and
pONY8Z CMVHyb. which had been prepared for ligation by digestion with the same
enzymes. These plasmids were designated pONY8ZA or pONY8ZA MLVHyb and
pONY8ZA CMVHyb. The sequence for pONY8ZA CMVHyb is provided in SEQ ID No
52. These plasmids have a unique *XbaI* site into which can be inserted genes such as

-55-

GFP or neomycin phosphotransferase or other biologically active entity. This use of the site is demonstrated for GFP. The GFP ORF was obtained from pEGFP-1 (Clontech) by digestion with SmaI and XbaI, and then the ends filled in by treatment with T4 DNA polymerase. This fragment was then ligated into pONY8ZA or pONY8ZA MLVHyb and pONY8ZA CMVHyb prepared for ligation by digestion with XbaI and subsequent filling in with T4DNA polymerase. The resulting vector plasmids were called pONY8GZA or pONY8GZA MLVHyb and pONY8GZA CMVHyb. Other genes can be inserted at this site by manipulations apparent to those skilled in the art.

10 Creation of EIAV vector genomes containing loxP sites in their LTR's

The time taken to construct producer cell lines for EIAV vectors would be greatly reduced if it was possible to 1) locate and 2) reutilise a site in the host cell chromosome which was particularly favourable for high levels of transcription of the vector genome. In outline, this can be achieved by engineering loxP sites in the 3'LTR of EIAV vectors, transduction of the packaging cell line with vectors which carry loxP and hybrid LTRs, selection of cells which express the highest levels of vector genome and exchange of the test EIAV vector genome for the vector genome of choice using the cre/loxP recombination system.

20

The proposed scheme was evaluated using a derivative of pONY8GZA CMVHyb in which a loxP site was introduced into the PstI site between the EIAV sequences (required for efficient integration) and the CMV promoter in the 3'LTR. After transduction the integrated vector will thus have a loxP-CMV cassette located in the 5'LTR and 3'LTR's and therefore full length transcripts of the vector genome will be driven by the CMV promoter, which is a powerful promoter. pONY8GZA CMVHyb contains many PstI sites hence it was modified to allow insertion of the loxP site by digestion with XbaI and NheI and religation to create the subclone, pONY CMVHyb. This plasmid has a unique PstI site in the hybrid LTR. The loxP site was inserted into this site using two complimentary oligonucleotides which when annealed formed PstI-compatible termini. These were termed loxP POS

-56-

PSTI [GATAAC TTCGTATAATGTATGCTATACGAAGTTATCTGCA] (SEQ ID No 21)] and

loxP NEG PstI [GATAACTTCGTATAGCATACATTATACGAAGTTATCTGCA]
5 (SEQ ID No 22)

The sequence and orientation of the loxP site was confirmed by DNA sequencing and the plasmid called pONY CMVHyb loxP. The central part of the vector genome was then reintroduced into this subclone by transfer of the NotI-BstEII fragment from pONY8GZA
10 CMVHyb into pONY CMVHyb cut the same way. The resulting vector was termed pONY8GZA CMVHyb loxP.

Two routes for construction of a producer cell line are available using pONY8GZA CMVHyb loxP. The plasmid can be introduced into a packaging cell line by transfection
15 or vector particles can be made using the 293T and these used to transduce the packaging cell line. Since the vector is derived from EIAV, rather than MLV, it is able to transduce non-dividing cells or slowly dividing cells. In this situation it has been hypothesised that integrations occur at chromosomal sites that are constitutively open; that is, are likely to be sites at which high levels of transcription will be maintained for extended periods.
20 This may be important for the long term usefulness of the producer cell line and thus represents an advantage of strategy using transduction.

Producer cell lines were made by transfection or transduction of a TE671-derived cell line (EV11E) which has stably integrated copies of VSV-G and the synthetic EIAV gag/pol
25 under the control of CMV promoters. Prior to transfection with pONY8GZA CMVHyb loxP it was linearised by digestion with AhdI. Seven days following transfection or transduction cells expressing the highest levels of GFP were selected by FACS and then cloned by limiting dilution. A number of clones were analysed for levels of full length vector RNA using Taqman technology based assays in order to confirm the hypothesis
30 that the highest level of GFP expression correlates with the highest levels of vector RNA.

The cell line which expressed the highest level of RNA was then tested for production of transducing vector particles 5 days after changing the temperature of incubation from 37C

-57-

to 32°C. At 32°C, VSV-G expression is induced however maximal levels of VSV-G are only obtained after 5 days at the permissive temperature (see WO 00/52188). The cell line producing the highest titre, EV11E CMV loxP was selected for further work.

- 5 In order to exchange the vector genome with for another EIAV vector genome with a more suitable configuration for use in the clinical setting EV11EloxP cells were transfected with cre recombinase expression plasmid, pBS185 (Gibco), which results in excision of the EIAV vector between the loxP sites. This leaves a loxP-CMV promoter R-U5 sequence in the cells. Cells from which the EIAV vector genome had been excised
10 were selected on the basis of low levels of GFP expression by FACS and assumed to be clonal on the basis of the clonality of EV11E CMV loxP. These were termed EV11EloxPΔ and used as targets for new EIAV vector genomes.

15 **Construction of EIAV vector genomes with downstream REV expression cassettes and flanking loxP sites**

The production of vector particles from minimal EIAV vectors (those which do not express EIAV REV or any other EIAV proteins) is increased by about 10-fold in the presence of EIAV REV in our 293T transient production system when the codon-
20 optimised EIAV gag/pol expression construct is used to drive production of vector particles as set out in Table 2. This may be improved nuclear to cytoplasmic transport of the vector genome in the presence of REV protein. Packaging/producer cell lines for EIAV vectors may be engineered to express Rev protein. One approach would be to engineer cells to express EIAV REV from an independently transfected expression
25 cassette. However, the cassette and the vector genome may be subject to differential regulation, for example by methylation or chromosome remodelling. Such an effect may limit the useful life of such cell lines.

30 **Table 2. Effect of REV expression on titres obtained from REV-expressing [REV+] and non-expressing [REV-] vectors**

vector plasmid	gag/pol	expression	#titre
----------------	---------	------------	--------

-58-

	plasmids	(l.f.u./ml)
pONY4Z [REV+]	pONY3.1	$2.0 \pm 0.4 \times 10^6$
pONY4Z [REV+]	pE SYN GP	$0.9 \pm 0.2 \times 10^6$
pONY8Z [REV-]	pONY3.1	$1.5 \pm 0.2 \times 10^6$
pONY8Z [REV-]	pE SYN GP	$1.9 \pm 0.6 \times 10^5$

* Titre was measured on D17 cells and is expressed as LacZ forming units/ml (l.f.u./ml). Transfections were carried out in 293T cells using the vector plasmid and gag/pol expression plasmid shown and pRV67 (VSV-G expression plasmid) (See WO 00/52188).

5

REV+ and REV- reflect the rev expression status of the vectors. REV+ reflects vectors which express the REV protein. REV- reflects expression vectors which do not express the REV protein.

10 **pESYNGP**

The gag/pol expression plasmid shown in Figure called called pESYNGP was constructed as follows: The codon-optimised EIAV gag/pol ORF was synthesised by Operon Technologies Inc., Alameda and supplied in a proprietary plasmid backbone, GeneOp. The complete fragment synthesised included sequences flanking the EIAV gag/pol ORF: tctagaGAATTCGCCACCATG- **EIAV gag/pol**- UGAACCCGGGgcgccgc (SEQ ID No 44). The ATG start and UGA stop codons are shown in bold. XbaI and NotI sites are in lower case. These were used to transfer the gag/pol ORF from GeneOp into pCIneo (Promega) using the NheI and NotI sites in the latter.

20

pESDSYNGP

An alternative expression plasmid for expression of the synthetic EIAV gag/pol could also be used. It is called pESDSYNGP and its construction is described as follows: ESDSYNGP was made from pESYNGP by exchange of the 306bp EcoRI-NheI fragment, from just upstream of the start codon for gag/pol to approximately 300 base pairs inside

25

-59-

the gag/pol ORF with a 308bp EcoRI-NheI fragment derived by digestion of a PCR made using pESYNGP as template and using the following primers: SD FOR [GGCTAGAGAATTCCAGGTAAGATGGGCGATCCCCTCACCTGG] (SEQ ID No 60) and SD REV [TTGGGTACTCCTCGCTAGGTTC] (SEQ ID No 61). This manipulation replaces the Kozak consensus sequence upstream of the ATG in pESYNGP with the splice donor found in EIAV. The sequence between the EcoRI site and the ATG of gag/pol is thus CAGGTAAG (SEQ ID No 62).

The sequences for pESYNGP (SEQ ID No 53) and pESDSYNGP (SEQ ID No 54) are provided.

Packaging/Producer cells may be engineered by physically linking the genome and EIAV REV expression cassettes. In this way stable transfectants may be generated which contain the vector genome and the EIAV REV expression cassette in the same chromatin environment. This manipulation may ensure that the relative levels of transcription of the vector genome and the REV expression cassette are maintained leading to an increased duration of vector production from the producer cells.

Previous work has suggested that optimisation of the level of REV may be required with respect to the level of vector genome (see WO 98/17815). We have examined the levels of vector production in a transient system in which several different promoters are used to drive REV expression in order to determine which vector genome-rev expression cassette is optimal for use in constructing producer cell lines. The highest titres were obtained with FB29 and PGK promoters driving REV expression.

The following describes the construction of EIAV vector genomes plasmids in which there is a downstream expression cassette for synthetic EIAV REV protein. The promoters tested were FB29, PGK, TK, CMV, SV40 and RSV. In addition the loxP sites were engineered into the vector plasmid backbone in such a way that the genome and introduced promoter-REV expression plasmid was flanked. In this way, the complete vector-REV cassette can be recombined into loxP sites in the target cell.

-60-

The complete construction of the FB29 and PGK containing plasmids is described here. The REV expression construct was inserted in the both orientations with respect to the EIAV vector genome. Plasmids in which the FB29 or PGK promoters drive REV expression are being utilised for construction of stable producer cell lines.

5

Construction of plasmids

In the first step of construction an SfiI site was inserted downstream of the EIAV vector sequence. This site is the insertion site for the promoter REV cassettes. The construction was made as follows. pONY8Z was digested with EheI and NruI, the ends were blunted by treatment with T4 DNA polymerase and religated. The resulting plasmid, pONY8Z delta, is thus deleted with respect to the leader, gag, reporter cassette and most of the Rev/RRE regions.

15 pONY8Z delta was mutated to contain loxP sites inserted in the DraII site immediately to the 5' of the CMV promoter and in the BspLU11I site to the 3' of the vector genome. The loxP sites were inserted using complementary nucleotide pairs which when annealed had overhanging termini suitable for cloning into these sites and were inserted in two steps of cloning. The oligonucleotides for insertion into the DraIII site were

20

VSAT 158: [GTGATAACTTCGTATAATGTATGCTATACGAAGTTATCACTAC]
(SEQ ID No 23)

and

25

VSAT 155 [GTGATAACTTCGTATAGCATACATTATACGAAGTTATCACGTA]
(SEQ ID No 24)

For the BspLU11I they were:

30 VSAT 156 [CATGTATAACTTCGTATAATGTATGCTATACGAAGTTATA] (SEQ ID
No 25) and

-61-

VSAT 157 [CATGTATAACTTCGTATAGCATACATTATACGAAGTTATA] (SEQ ID No 26)

Plasmids were selected in which the orientation of the loxP at both sites were the same
5 and the same as the EIAV vector genome. The modified plasmid was called pONY8Z delta 2xloxP.

pONY8Z delta 2xloxP has a unique PvuII site downstream of the deleted EIAV vector genome into which annealed complementary oligonucleotides encoding SfiI sites were
10 inserted. The oligonucleotides were:

SFI SRFPOS [AGTAGGCCGCTCGGCCGCCCCGGGCATCA] (SEQ ID No 27) and

SFI SRF NEG [TGATGCCCGGGCGGCCGAGGCGGCCTACT] (SEQ ID No 28)
15

Clones which had the SfiI – SrfI sites in either orientation were selected for further work. These were called pONY8Z delta SfiI FOR and REV.

Amplification and cloning of FB29 and PGK promoters

20

The FB29 promoter was amplified from pRDF (Cosset FL, et al. *J Virol* 1995 Dec;69(12):7430-6) using primers:

FB29 POS [TAGCCGAGATCTCAAATTGCTTAGCCTGATAGCC] (SEQ ID No 29)and

25 FB29 NEG [TGCCTAGCTAGCCTCCCGGTGGTGGGTCCGGTG] (SEQ ID No 30)
which introduce

5'BglII and 3'NheI sites.

The PGK promoter was amplified from pPE327 using primers

PGK POS [AGCAGTAGATCTGGGGTTGGGGTTGCGCCTTT] (SEQ ID No 31) and

30 PGK NEG [CGTCATGCTAGCCTGGGGAGAGAGGTCGGTG] (SEQ ID No 32)

-62-

The PGK promoter sequence obtained from this plasmid was the same as the sequence of GenBank Acc. No. M11958 except that it has a single mutation: nucleotide 347 of M11958 is changed from G to A. The TK promoter and intron was amplified from pRL-TK (Promega) with:

- 5 TK POS [TACGGAAGATCTAAATGAGTCTTCGGACCT] (SEQ ID No 33) and
TK NEG [CTCAACGCTAGCGTACTCTAGCCTTAAGAGCTG] (SEQ ID No 34)

The RSV promoter was amplified from pREP7 (Invitrogen) with

- 10 RSV POS [TACCAGAGATCTTCTAGAGTCGACCAATTCTCATG] (SEQ ID No 35)
and

RSV NEG [CATCGAGCTAGCAGCTTGGAGGTGCACACCAATG] (SEQ ID No 36)
and

15

The SV40 promoter was amplified from pCIneo (Promega) with:

SV40 POS [GATGGTAGATCTGCGCAGCACCATGGCCTGAA] (SEQ ID No 37) and

- 20 SV40 NEG [CTCGAAGCTAGCAGCTTTTTGCAAAAGCCTAGGC] (SEQ ID No 38)

- The PCR fragments were digested with BglII and NheI and ligated into pSL1180 (Pharmacia) which had been prepared by digestion using the same enzymes. Following transformation into E.coli DNA was prepared and the sequence of the promoters checked
25 by DNA sequencing. Clones in which the correct promoter sequence was present were used for further work and were called pSL1180-FB29, pSL1180-PGK, pSL1180-RSV, pSL1180-SV40, pSL1180-TK.

- In the next step the promoter fragments were positioned to drive transcription of synthetic
30 EIAV REV in pE syn REV. pE syn REV is a pCIneo based plasmid (Promega) which was made by introducing the EcoRI to SalI fragment from the synthetic EIAV REV plasmid into the polylinker region of the plasmid using the same sites. The synthetic

-63-

EIAV REV plasmid made by Operon contains a codon-optimised EIAV REV open reading frame flanked by EcoRI and SalI. The sequence of this fragment is shown as SEQ ID No 39.

- 5 Prior to replacement of the CMV promoter in pE syn REV it was modified as follows. The SV40 neo region was deleted by digestion with KpnI and BamHI, the ends blunted by treatment with T4 DNA polymerase and then religated. The plasmid was termed pE syn REV delta. Next SfiI sites were introduced into both the BglII site which is just 5' of the CMV promoter and DraIII site downstream of the polyA signal.

10

The oligonucleotides used for this were as follows:

SFI FOR BglII POS [GATCGGCCCGCCTCGGCCA] (SEQ ID No 40) and

- 15 SFI FOR BglII NEG [GATCTGGCCGAGGCGGCC] (SEQ ID No 41)and

SFI FOR DRA POS [GGCCGCCTCGGCCGTA] (SEQ ID No 42) and

SFI FOR DRA NEG [GGCCGAGGCGGCCTAC] (SEQ ID No 43)

20

- Clones in which the SfiI was located 5' of the BglII site were selected were used for further work. The plasmid obtained after this two step manipulation was termed pE syn REV delta 2xSfiI. It has the following features: 5'SfiI sites – BglII site - CMV promoter and intron – NheI site – E syn REV - polyA site – 3'SfiI site. Hence the CMV promoter
- 25 can be excised by digestion with BglII and NheI and replaced with the promoter of choice obtained from the pSL1180 series of clones by digestion with the same enzymes. Construction details are included from this point for only the constructs which contained FB29 and PGK promoters, however a similar scheme was used for the other promoters, except that a partial SfiI digestion was required for transfer of the SV40-REV cassette.

30

Promoter fragment were obtained from pSL1180 – FB29 and pSL1180 – PGK by digestion with BglII and NheI and ligated into pE syn REV delta 2xSfiI digested with the

-64-

same enzymes. The resulting plasmids were called FB29 E SYN REV and PGK E SYN REV.

In the next stage the internal regions of pONY8G, pONY8.1G SIN MIN and pONY4G were obtained by digestion with SgfI (which has unique site in the CMV promoter driving the EIAV vector genome) and MunI (which cuts in the 3'LTR) and ligated in to pONY8Z delta SfiI FOR and REV prepared for ligation by digestion with the same enzymes. The resulting plasmids were called pONY8G SfiI FOR and REV, pONY8.1G SIN MIN SfiI FOR and REV and pONY4G SfiI FOR and REV.

In the final stage the promoter-REV cassettes were moved from FB29 E SYN REV and PGK E SYN REV into pONY8G SfiI FOR and REV, pONY8.1G SIN MIN SfiI FOR and REV and pONY4G SfiI FOR. This manipulation was achieved as follows. FB29 E syn REV, PGK E syn REV, and the vector plasmids described immediately above were digested with SfiI and ligations set up with appropriate fragments. The promoter-REV cassettes were orientated in the same or opposite orientations with respect to the EIAV vector genome in the 'FOR' and 'REV' plasmids. The resulting plasmids were called pONY8.3G FB29 + or - and pONY8.3G PGK+ and -. A schematic structure of the pONY 8.3 +/- plasmids is shown in Figure 17.

The performance of these constructs was tested in relation to pONY8G in 293T transient production assays and the results are shown in Table 3.

The sequence of the *EcoRI* to *SaII* fragment representing the codon-optimised EIAV REV open reading frame obtained from the plasmid synthesised by Operon (SEQ ID No 39)

EcoRI and *SaII* sites are in bold. The ATG start and UGA termination codons are underlined

GAATTCGCCACCATGGCTGAGAGCAAGGAGGCCAGGGATCAAGAGATG

-65-

ACCTCAAGGAA
 GAGAGCAAAGAGGAGAAGCGCCGCAACGACTGGTGGGAAGATCGACCCA
 AAGGCCCCCTG
 GAGGGGGACCAGTGGTGCCGCGTGCTGAGACAGTCCCTGCCCCGAGGAGAAGATTCTCT
 5 AGC
 CAGACCTGCATCGCCAGAAGACACCTCGGCCCCGGTCCCACCCAGCACACACCCTCC
 AGA
 AGGGATAGGTGGATTAGGGGCCAGATTTTGCAAGCCGAGGTCCTCCAAGAAAGGCTG
 GAA
 10 TGGAGAATTAGGGGCGTGCAACAAGCCGCTAAAGAGCTGGGAGAGGTGAATCGCGG
 CATC
 TGGAGGGAGCTCTACTTCCGCGAGGACCAGAGGGGGCGATTTCTCCGCATGGGGAGGC
 TAC
 CAGAGGGCACAAGAAAGGCTGTGGGGCGAGCAGAGCAGCCCCCGCGTCTTGAGGCC
 15 CGGA
 GACTCCAAAAGACGCCGCAAACACCTGTGAAGTCGAC

Table 3

- 20 Titres obtained from a representative experiment in which the vector-REV constructs were tested by transient 293T production assay. The vector constructs were cotransfected with pE synGP, the synthetic EIAV gag/pol expression plasmid, and pRV67, VSV-G expression plasmid. Titres were measured in D17 cells.

Plasmid	Titre (g.f.u./ml)
pONY8G SfiI FOR	1.6×10^4
#pONY8G SfiI FOR PLUS pE syn REV	5.2×10^5
pONY8.3G FB29 +	8.8×10^5
pONY8.3G FB29 -	7.8×10^5
pONY8.3G PGK +	1.2×10^6
pONY8.3G PGK -	1.2×10^6
pONY8G	9.4×10^5

Titre was assessed on D17 cells and is expressed as green fluorescent protein cell units/ml (g.f.u./ml). Transfections were carried out with pE syn GP KOZAK and pRV67 as described previously.

- 5 * pONY8G SfiI FOR is identical to the pONY8.3 derivatives except that there is no promoter-REV expression cassette is inserted in the SfiI site

pE syn REV plasmid was also included in this transfection

- 10 pONY8G is a standard EIAV vector genome used for comparative purposes

pONY8.3G FB29 – is shown as SEQ ID No 45

pONY8.3G FB29 + is shown as SEQ ID No 46

- 15 pONY8.3GPGK – is shown as SEQ ID No 47

pONY8.3G PGK + is shown as SEQ ID No 48.

SUMMARY

20

Thus, in summation, the present invention provides high titer regulated retroviral vectors. These regulated retroviral vectors include lentivectors, HRE-regulated vectors and functional SIN vectors which can be produced at high titres from derived producer cell lines.

25

The present invention also provides a method other than retroviral transduction for the transfer of a regulated retroviral vector into a derived producer cell line. This method comprises a recombinase assisted method which allows for the production of high titer regulated retroviral vectors.

30

In one broad aspect, the present invention relates to the selection of cells which express high levels of a retroviral vector genome and exchange of this retroviral genome for the

-67-

vector genome of choice, preferably a regulated retroviral vector genome or a lentiviral vector genome using a cre/loxP recombination system. Thus, the present invention enables regulated retroviral vectors to be produced at high titres from transduced producer cell lines.

5

In another broad aspect, the present invention relates to the selection of cells which express high levels of a retroviral vector genome and exchange of this retroviral genome for the vector genome of choice, preferably a regulated retroviral vector genome or a lentiviral vector genome using a cre/loxP recombination system and a retroviral vector
10 production system which incorporates a REV protein production system. Thus, the present invention enables regulated retroviral vectors to be produced at high titres from transduced producer cell lines.

All publications mentioned in the above specification are herein incorporated by
15 reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various
20 modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A method of modifying a producer cell which producer cell comprises integrated into its genome a provirus which provirus comprises one or more recombinase
5 recognition sequences within or upstream of its 3' LTR, the method comprising:
introducing into the cell a construct comprising a 5' recombinase recognition sequence,
an LTR and a 3' recombinase recognition sequence in that order, in the presence of a
recombinase which is capable of acting on the recombinase recognition site(s) such that
the nucleotide sequence between the 5' and 3' recombinase recognition sequences in the
10 construct is introduced into the provirus.
2. A method according to claim 1 wherein the construct further comprises at least
one nucleotide sequence of interest (NOI) between the 5' recombinase recognition
sequence and the LTR, which NOI is operably linked to a transcriptional regulatory
15 sequence.
3. A method according to claim 1 or claim 2 wherein the construct further comprises
a 5'LTR and/or a packaging signal.
- 20 4. A method according to any one of claims 1 to 3 wherein the LTR is a
heterologous regulatable LTR.
5. A method according to claim 4 wherein the regulatable LTR comprises an
ischaemic like response element (ILRE).
25
6. A method according to any one of claims 1 to 3 wherein the LTR is inactive.
7. A method according to any one of the preceding claims wherein the provirus
comprises an NOI encoding a selectable marker, which NOI is flanked by recombinase
30 recognition sites
8. A method according to any one of the precedings claims wherein the provirus
comprises an internal 5' LTR upstream of the recombinase site or the 5' recombinase site

where there is more than one site.

9. A method according to any one of the preceding claims wherein the U3 region of the 5' LTR is inactive.

5

10. A method according to any one of the preceding claims wherein the U3 region of the 5' LTR and/or the U3 region of the second internal 5'LTR comprises a heterologous promoter.

10 11. A method according to any one of the preceding claims wherein the provirus comprises two recombinase recognition sites and as a preliminary step, the recombinase is expressed in a host cell such that the nucleotide sequence present between the two sites is excised.

15 12. A method according to any one of the preceding claims wherein the producer cell is a high titre producer cell.

13. A method according to any one of the preceding claims wherein the provirus is a lentivirus.

20

14. A method according to claim 13 wherein the lentivirus is HIV or EIAV.

15. A method according to any one of claims 2-14 wherein the provirus further comprises a second NOI.

25

16. A producer cell obtainable by the method of any one of claims 1 to 15.

17. An infectious retroviral particle obtainable from the producer cell of claim 16.

30 18. A derived producer cell comprising integrated into its genome a retroviral vector comprising in the 5' to 3' direction a first 5' LTR; a second NOI operably linked to a second regulatable 3' LTR; and a third 3'LTR;

-70-

wherein the third 3'LTR is positioned downstream of the second regulatable 3'LTR in the producer cell.

19. A producer cell according to claim 18 wherein the first 5' LTR comprising 5'R
5 and 5' U5 sequences is derivable from a first vector; the second NOI operably linked to a
second regulatable 3' LTR is derivable from a second vector; and the third 3'LTR is
derivable from the first vector.

20. A producer cell according to claim 18 or claim 19 wherein the first vector
10 comprises a retroviral vector wherein the retroviral vector comprises a first NOI flanked
by recombinase recognition sequences.

21. A producer cell according to claim 19 or claim 20 wherein the retroviral vector
further comprises an internal LTR located upstream of the first NOI and downstream of a
15 packaging signal wherein the internal LTR comprises a heterologous U3 sequence linked
to heterologous R and U5 sequences.

22. A producer cell according to any one of claims 18 to 21 wherein the third 3'LTR
is transcriptionally quiescent.

20

23. A producer cell according to claim 22 wherein the third 3' LTR comprises a
deletion in the U3 sequence.

24. A producer cell according to any one of claims 18 to 23 wherein the first NOI is a
25 selectable marker.

25. A producer cell according to claim 19 wherein the second vector comprises a
second NOI operably linked to a second regulatable 3'LTR comprising at least one
recombinase recognition sequence.

30

26. A producer cell according to 25 wherein the second regulatable 3'LTR comprises
a deletion in the U3 sequences in the 3'LTR.

-71-

27. A producer cell according to claim 25 or claim 26 wherein the second NOI comprises a coding sequence operably linked to a promoter.

28. A producer cell according to claim 27 wherein the second NOI comprises a
5 discistronic construct.

29. A producer cell according to claim 28 wherein the discistronic construct comprises a therapeutic gene, an internal ribosomal entry site (IRES) and a reporter gene.

10 30. A method for producing a high titre regulatable retroviral vector, the method comprising the steps of:

(i) providing a derived producer cell comprising integrated into its genome a first vector;

15 (ii) introducing a second vector into the derived producer cell using a recombinase assisted method;

wherein the derived producer cell comprises a retroviral vector comprising in the 5' to 3' direction a first 5' LTR; a second NOI operably linked to a second regulatable 3' LTR;
20 and a third 3'LTR; wherein the third 3'LTR is positioned downstream of the second regulatable 3'LTR in the derived producer cell.

31. A method according to claim 30 wherein the third 3' LTR is transcriptionally active but expression is directed away from the second regulatable 3'LTR.

25

32. A method for introducing a second regulatable 3'LTR into a derived producer cell wherein the method comprises a recombinase assisted method.

33. A method according to claim 32 wherein the recombinase assisted method is a
30 Cre/lox recombinase method.

-72-

34. A process for preparing a regulated retroviral vector as defined in claim 17 comprising performing the method according to any one of claims 30 to 33 and preparing a quantity of the regulated retroviral vector.
- 5 35. A regulated retroviral vector produced by the process according to claim 34.
36. A regulated retroviral vector according to claim 35 wherein the retroviral vector is capable of transducing a target site.
- 10 37. A regulated retroviral vector according to claim 36 wherein the retroviral vector is produced in sufficient amounts to effectively transduce a target site.
38. A regulated retroviral vector according to claim 36 or claim 37 wherein the target site is a cell.
- 15 39. A cell transduced with a regulated retroviral vector according to claim 38.
40. Use of a regulated retroviral vector according to any one of claims 35 to 38 in the manufacture of a pharmaceutical composition to deliver an NOI to a target site.
- 20 41. Use of a regulated retroviral vector according to any one of claims 35 to 38 in the manufacture of a medicament for diagnostic and/or therapeutic and/or medical applications.
- 25 42. Use of a recombinase assisted mechanism to introduce a regulated 3'LTR into a derived producer cell line to produce a high titre regulated retroviral vector.
43. A derived stable producer cell capable of expressing regulated retroviral vectors according to claims 35 to 38.
- 30 44. A derived stable producer cell according to claim 43 wherein the regulated retroviral vector is a high titre regulated retroviral vector.

-73-

49. A nucleic acid molecule according to any one of claims 46 to 48 wherein the LTR is a heterologous regulatable LTR.

50. A nucleic acid molecule according to any one of claims 46 to 48 wherein the LTR
5 is transcriptionally quiescent.

51. A method and/or a producer cell substantially as described herein and with reference to the accompanying Figures.

10

15

20

25

30

35

40

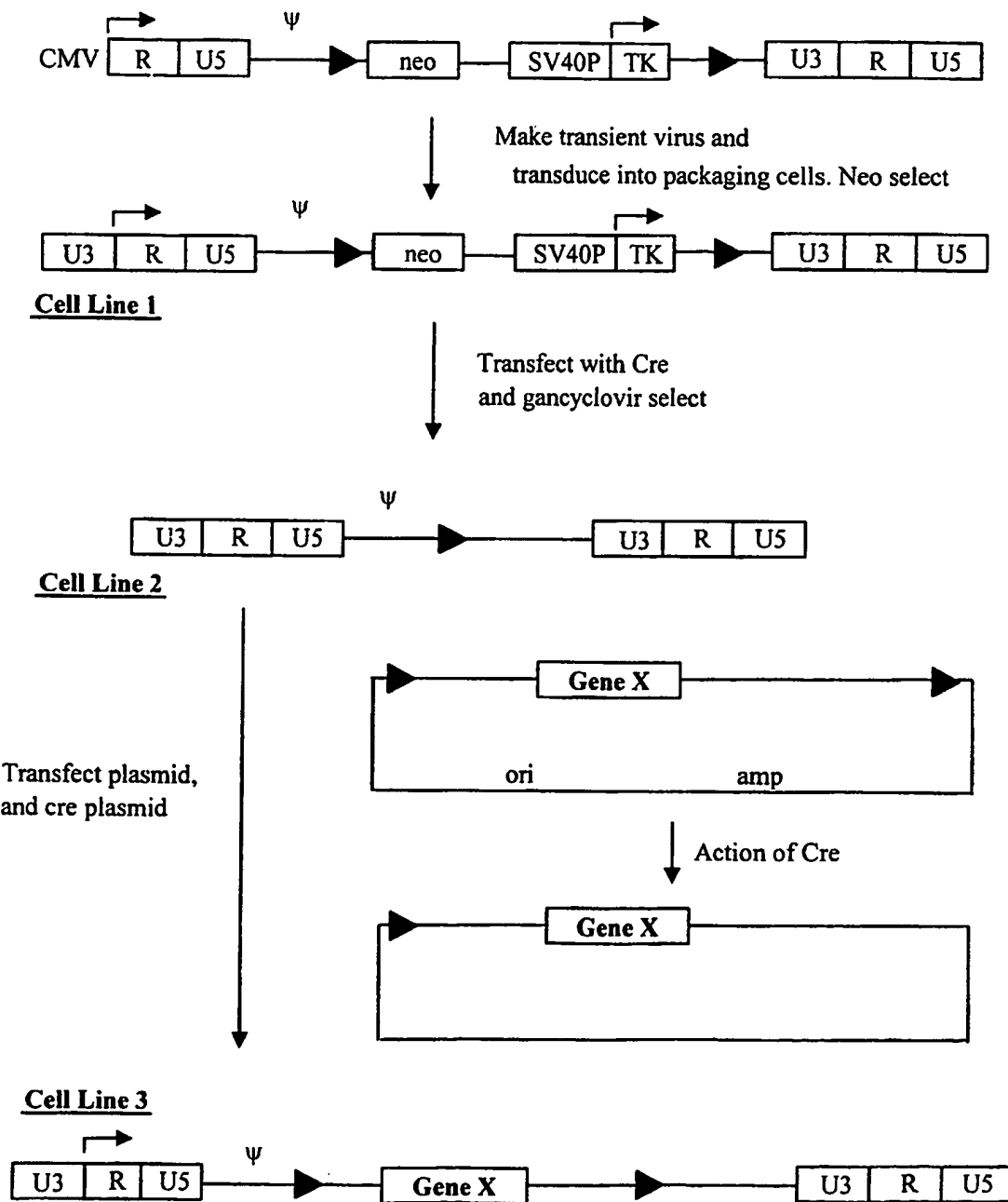
45

50

1 / 16

FIG. 1

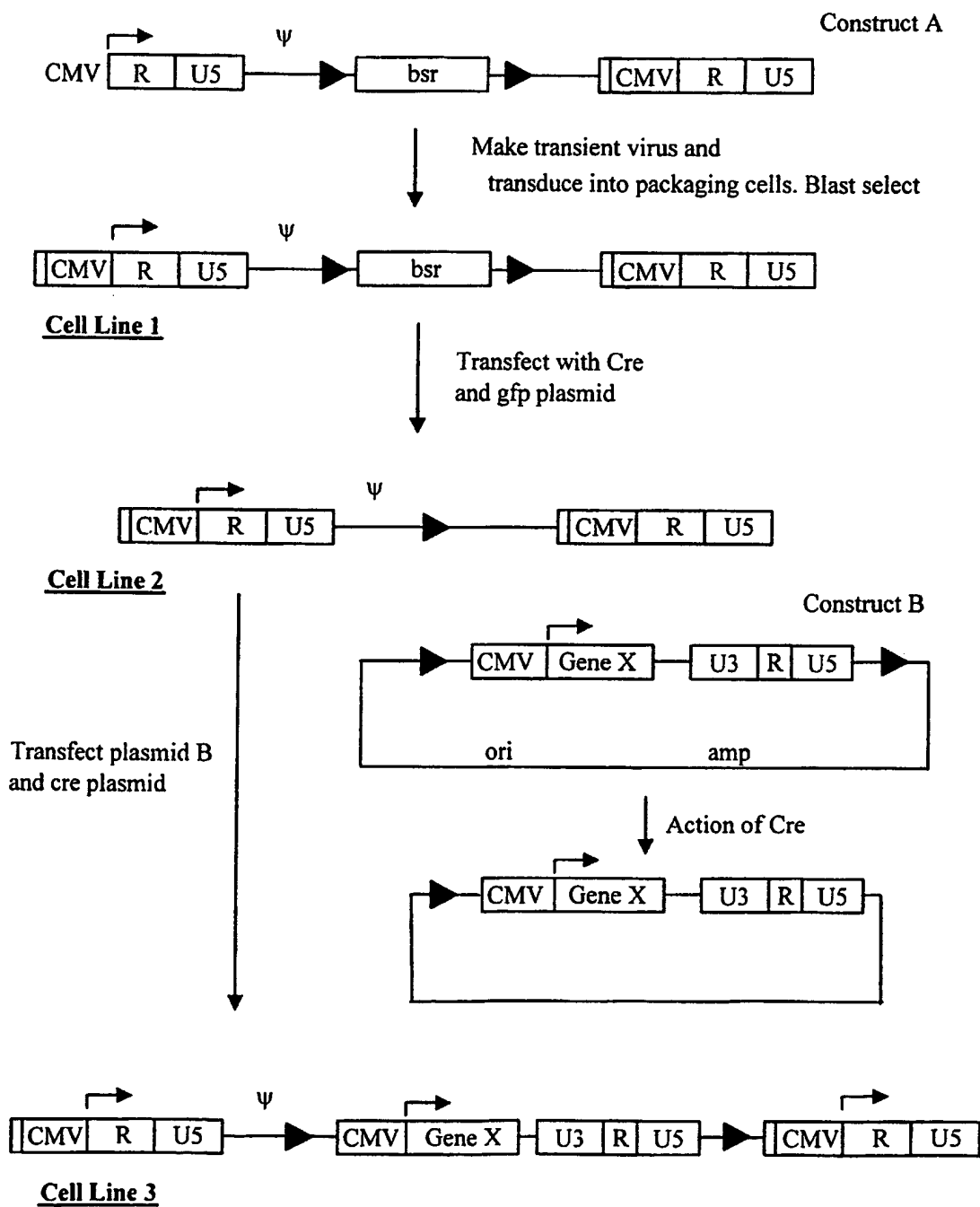
MLV-based transduction using Cre/loxP system as previously described



2 / 16

FIG. 2

EIAV-based transduction Cre/loxP system



3 / 16

FIG. 3

MLV SIN vector approach, with EIAV components in blue

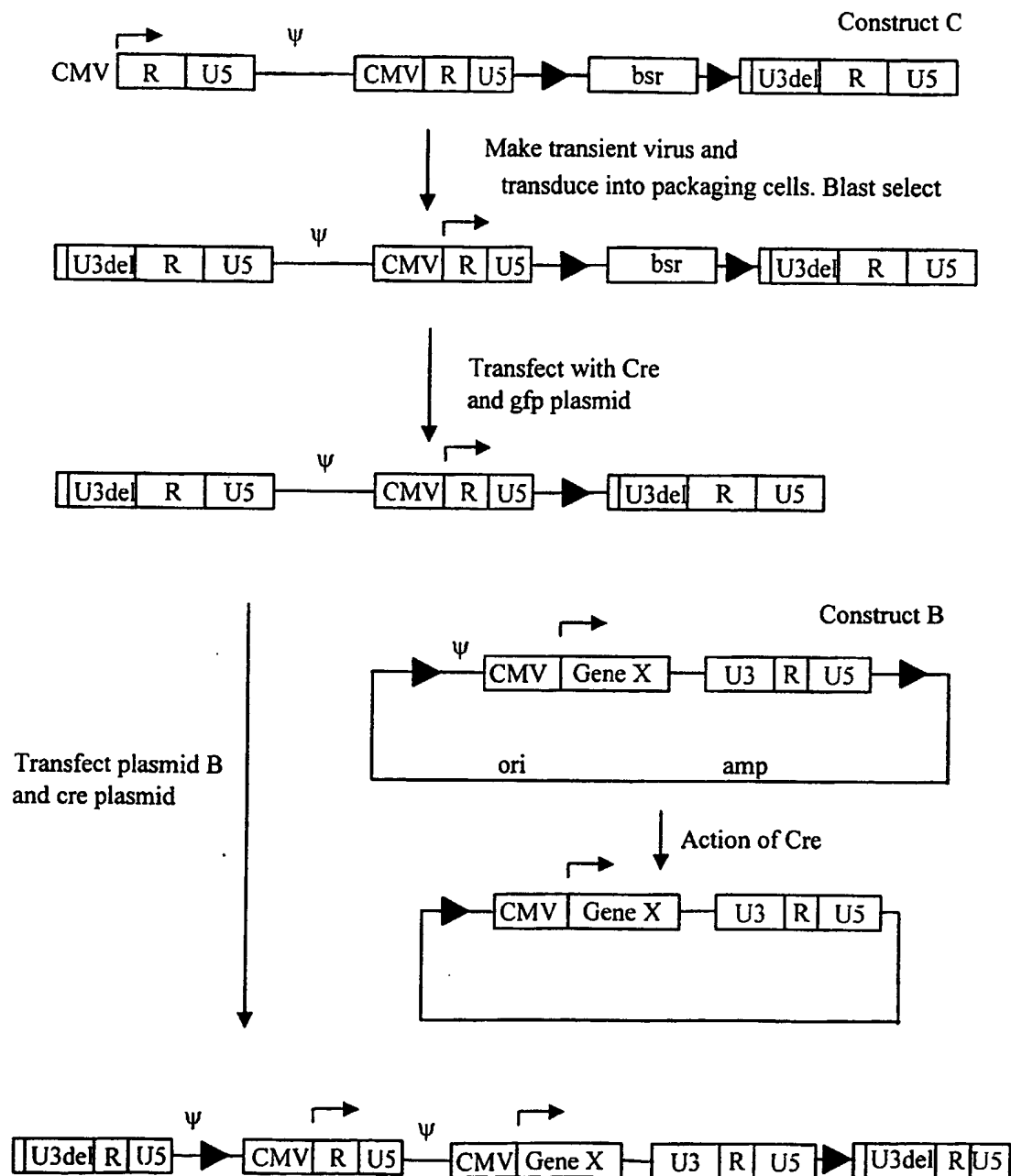
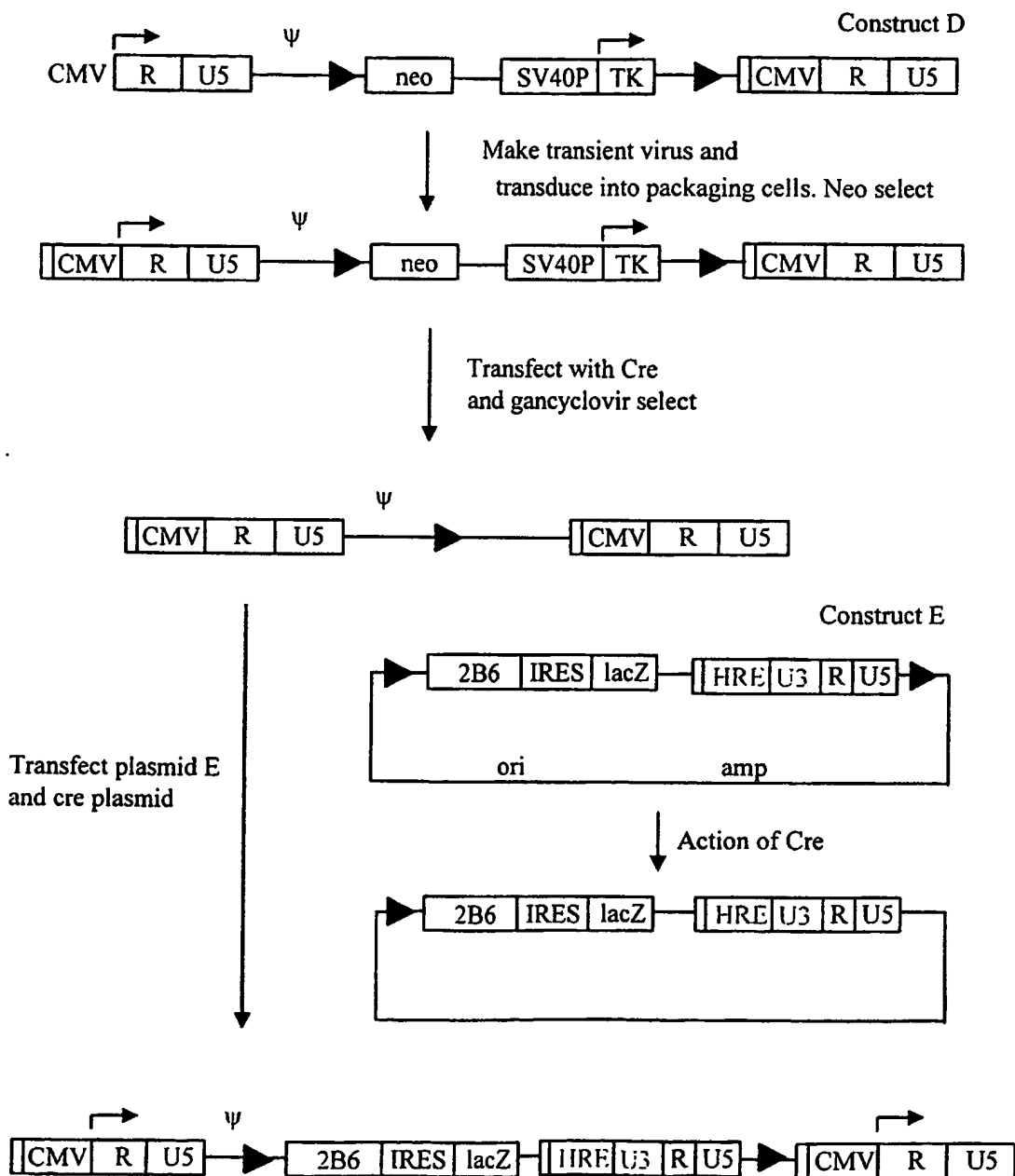


FIG. 4

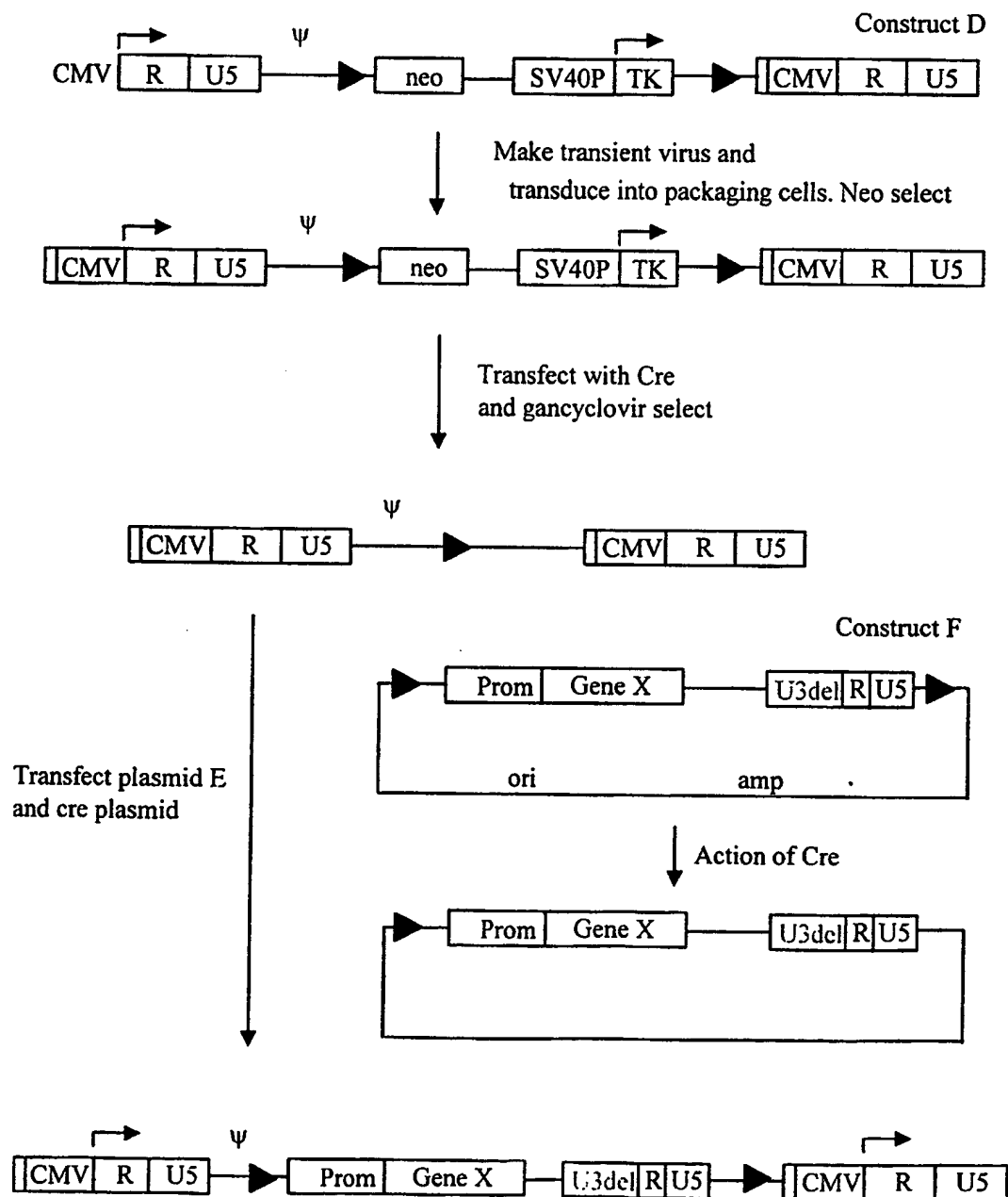
MLV-based transduction with HRE 3' LTR using Cre/loxP system



5 / 16

FIG. 5

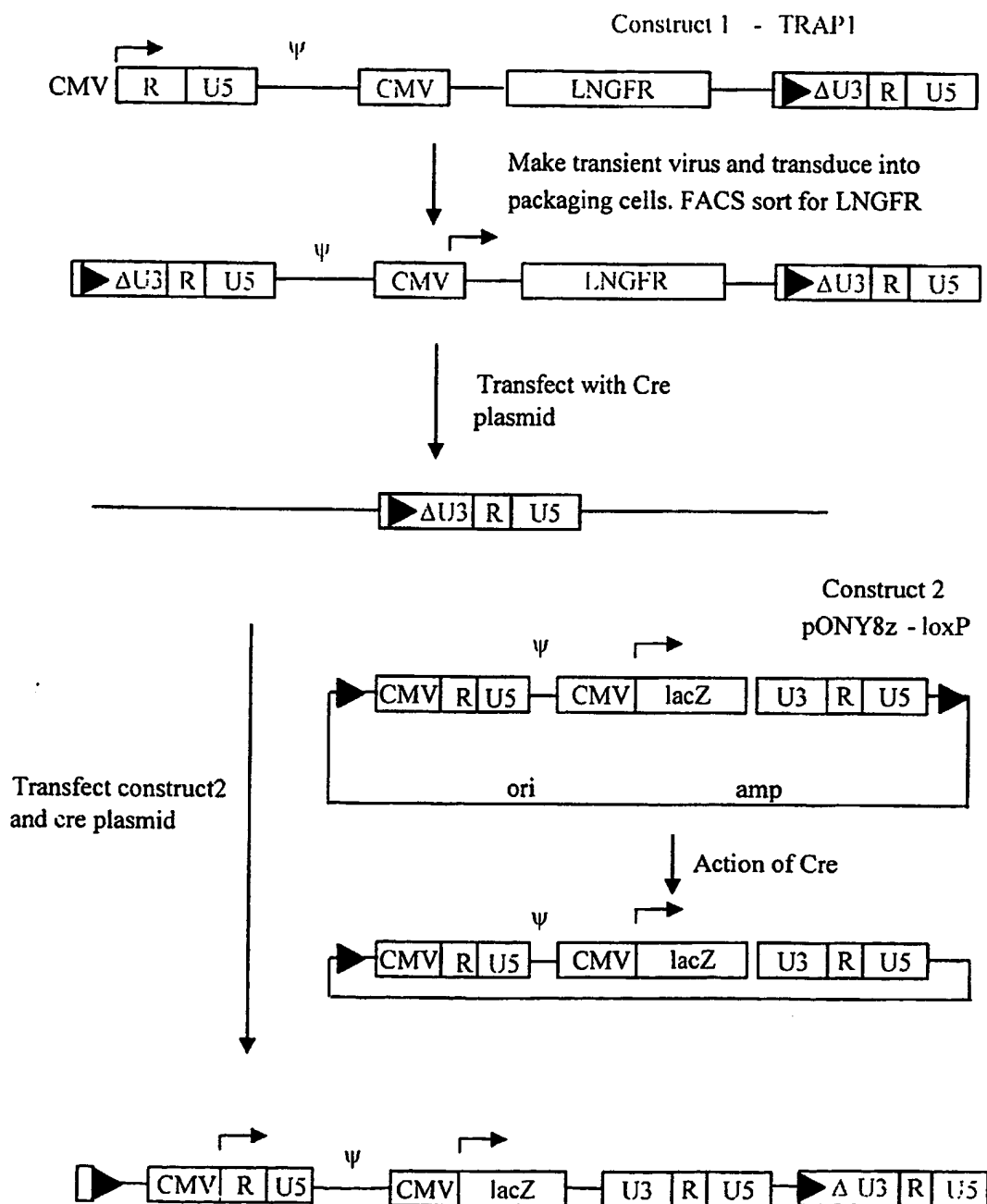
MLV-based transduction for SIN vector production using Cre/loxP system



6 / 16

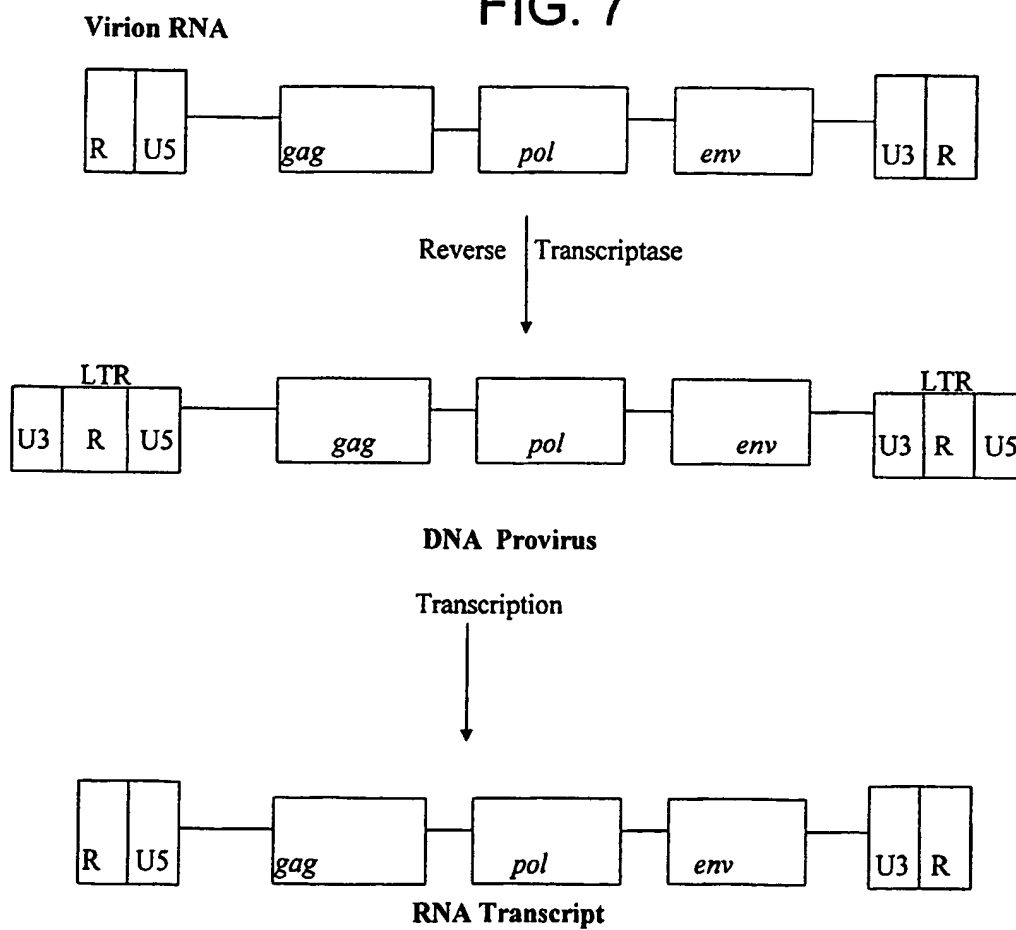
FIG. 6

MLV SIN-vector based transduction system. This general approach can be used with EIAV, HIV or MLV genomes



7 / 16

FIG. 7



8 / 16

FIG. 8

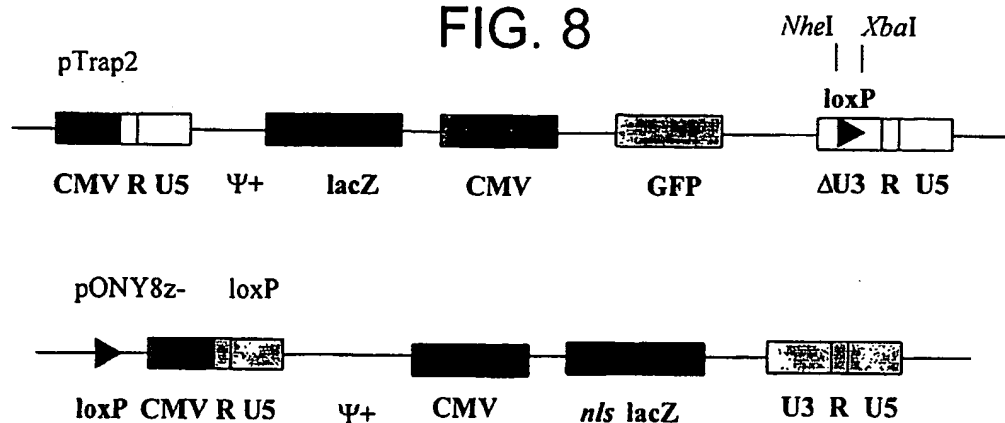
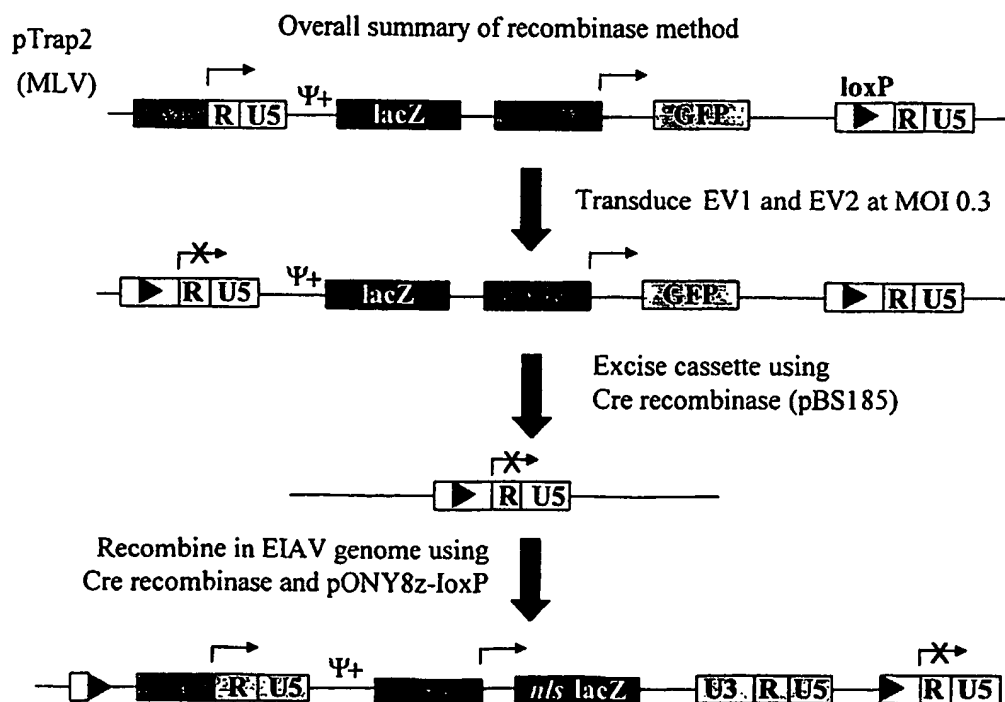
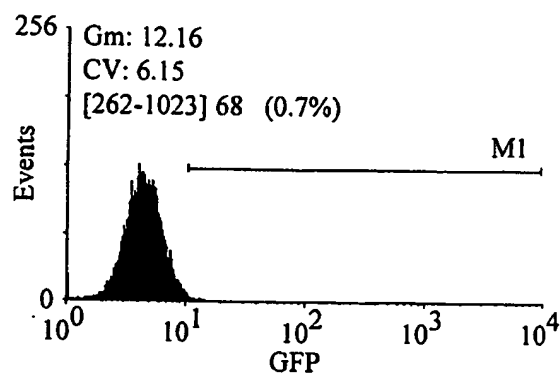


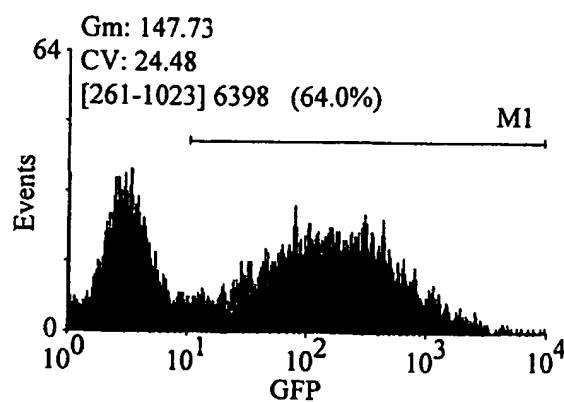
FIG. 9



9 / 16

**FIG. 10a**

FACS analysis of EV1 packaging
cells prior to transduction with
Trap2 vector

**FIG. 10b**

FACS analysis of EV1 packaging
cell line transduced with Trap2 at an
MOI of 0.3. A 5% top-slice of the
highest expressers was carried out

10 / 16

FIG. 11

Validation of the $\Delta\Delta C_t$ method for quantitation of GFP mRNA, relative to β -actin.

A titration of total RNA from EV1 clone A was used. The difference in C_t values between the two assays is shown on the y-axis. The magnitude of the gradient must be <0.1 for the method to be valid. The gradient is 0.077, so the method is suitable.

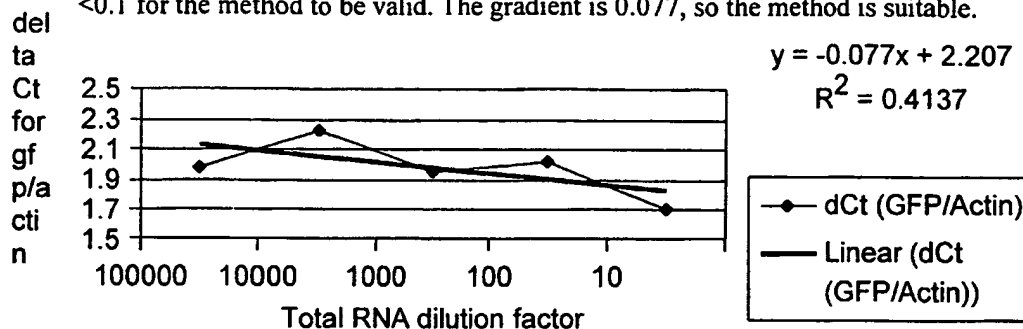
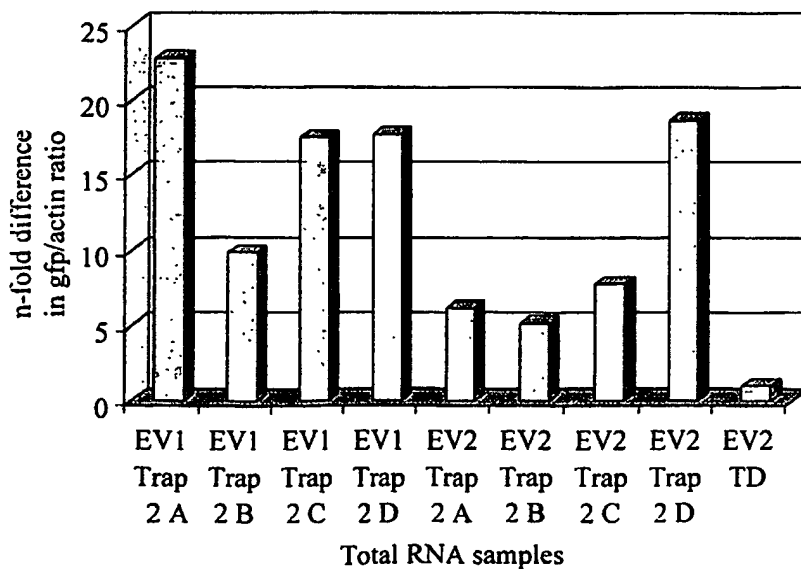


FIG. 12

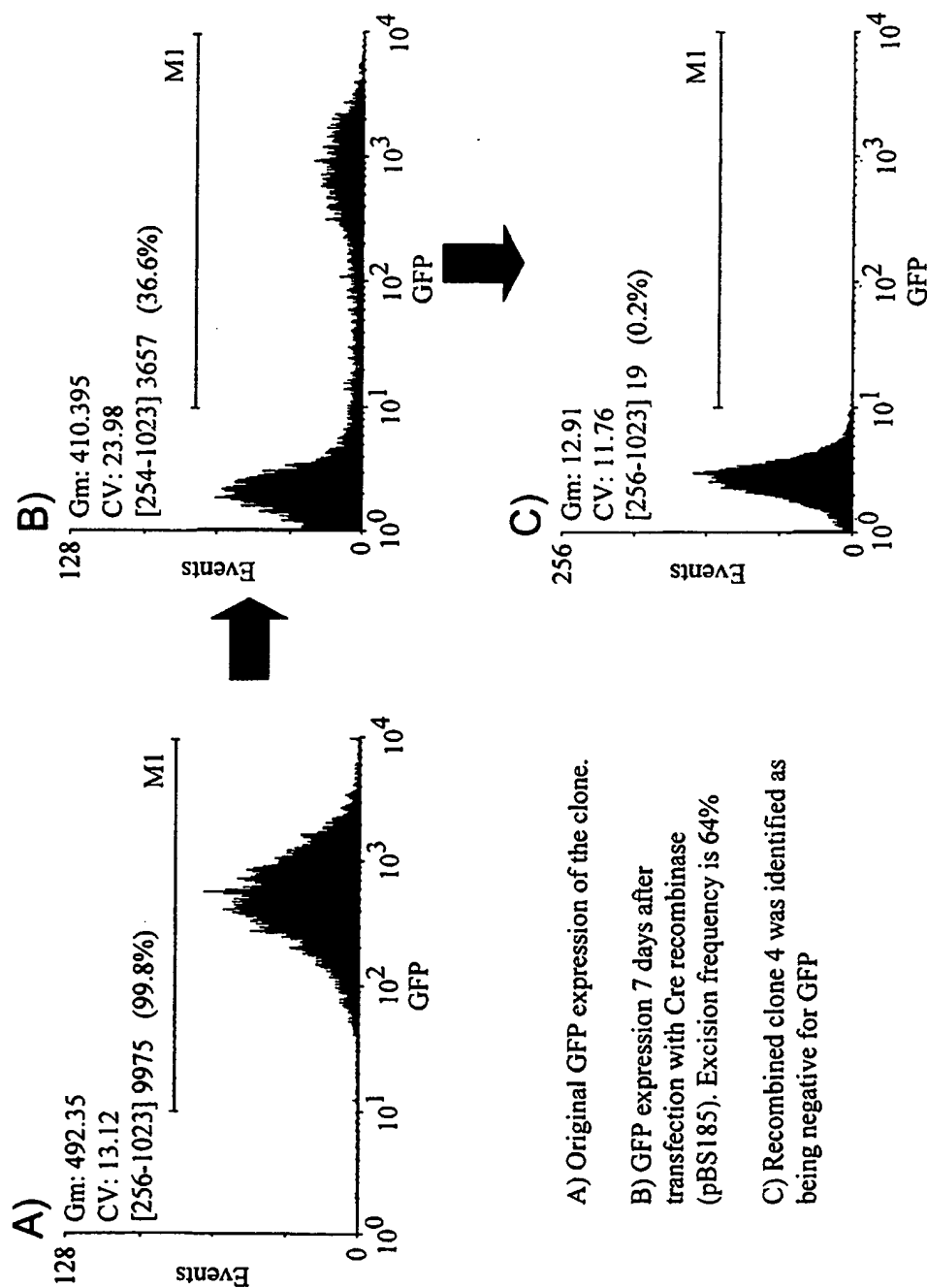
Quantitation of GFP mRNA relative to control β -actin mRNA. EV2 TD cells are transduced with Trap2 at an MOI of 0.3 and are the calibrator sample with the ratio designated 1.0.

Comparison of GFP expression levels in recombinase cell lines



11 / 16

FIG. 13





EV1 A4 cre/pONY8Z



EV2 D4 cre/pONY8Z



EV1 A4 pONY8Z



EV2 D4 pONY8Z

FIG. 14

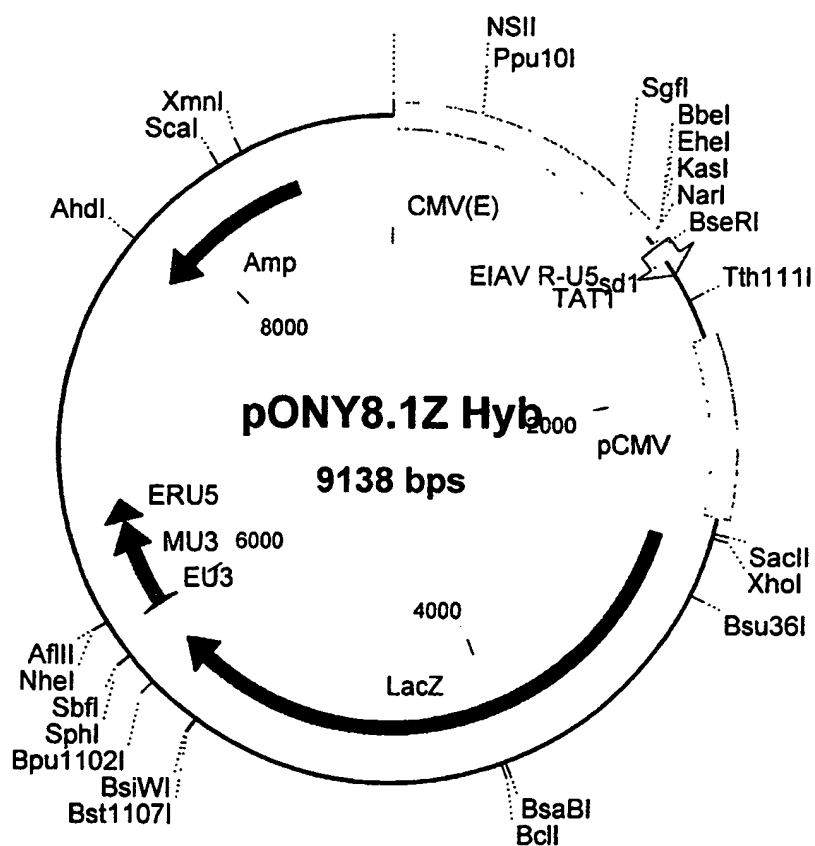


FIG. 15

14 / 16

FIG. 16

Alignment of leader and gag regions present in vectors pONY4Z, 8Z and ATG mutated 8Z vector. The later is referred to as pONY8ZA. The sequence aligned are from the NarI site in the leader to the XbaI site between the ELAV gag sequence and the CMV promoter. Sequences in the leader are shown in *italic* and a space is present upstream of the position of the gag ATG.

4Z	1 <i>cgcccgaacaggggacctgagagggg</i> gcagaccctacctgttgaacctgg
8Z	1 <i>cgcccgaacaggggacctgagagggg</i> gcagaccctacctgttgaacctgg
mutated 8Z	1 <i>cgcccgaacaggggacctgagagggg</i> gcagaccctacctgttgaacctgg
4Z	51 <i>ctgatcgtaggatccccgggacagcagaggagaacttacagaagtccttct</i>
8Z	51 <i>ctgatcgtaggatccccgggacagcagaggagaacttacagaagtccttct</i>
mutated 8Z	51 <i>ctgatcgtaggatccccgggacagcagaggagaacttacagaagtccttct</i>
4Z	101 <i>ggagggtgttcctggccagaacacaggaggacaggtaag</i> .at-gggagaccc
8Z	101 <i>ggagggtgttcctggccagaacacaggaggacaggtaag</i> .attgggagaccc
mutated 8Z	101 <i>ggagggtgttcctggccagaacacaggaggacaggtaag</i> .attgggagaccc
4Z	150 ttgacat-ggagcaaggcgctcaagaagttagagaagggtgacggtacaa
8Z	151 ttgacattggagcaaggcgctcaagaagttagagaagggtgacggtacaa
mutated 8Z	151 ttgacattggagcaaggcgctcaagaagttagagaagggtgacggtacaa
4Z	199 gggctcagaaattaactacttgtaactgtaattgggcgctaagtctagt
8Z	201 gggctcagaaattaactacttgtaactgtaattgggcgctaagtctagt
mutated 8Z	201 gggctcagaaattaactacttgtaactgtaattgggcgctaagtctagt
4Z	249 agacttatttcacat-gataccaactttgtaaagaaaaggactggcagctg
8Z	251 agacttatttcacat-gataccaactttgtaaagaaaaggactggcagctg
mutated 8Z	251 agacttatttcattgataccaactttgtaaagaaaaggactggcagctg

15 / 16

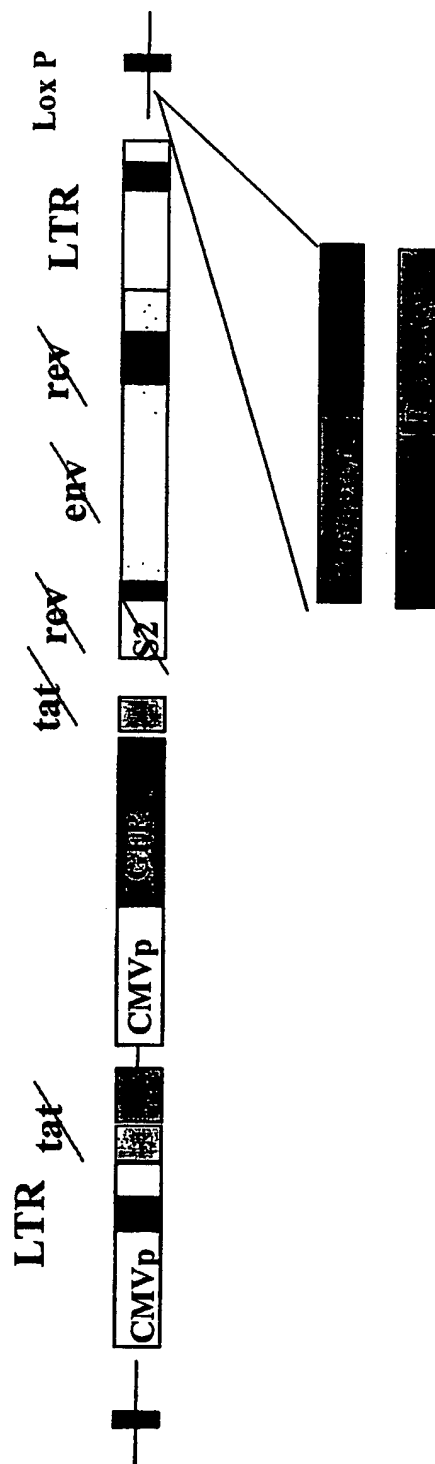
4Z	298 agggat-gtcattccattgctggaagat-gtaactcagacgctgtcagga
8Z	300 agggat-gtcattccattgctggaagat-gtaactcagacgctgtcagga
mutated 8Z	301 agggattgctattccattgctggaagattgtaactcagacgctgtcagga
4Z	346 caagaaagagaggcctttgaaagaacat-ggtgggcaatttctgctgtaa
8Z	348 caagaaagagaggcctttgaaagaacat-ggtgggcaatttctgctgtaa
mutated 8Z	351 caagaaagagaggcctttgaaagaacattggtgggcaatttctgctgtaa
4Z	395 agat-gggcctccagattaataat-gtagtagat-ggaaaggcatcattc
8Z	397 agat-gggcctccagattaataat-gtagtagat-ggaaaggcatcattc
mutated 8Z	401 agattgggcctccagattaataattgtagtagattggaaaggcatcattc
4Z	442 cagctcctaagagcgaaatat-gaaaagaagactgctaataaaaagcagt
8Z	444 cagctcctaagagcgaaatat-gaaaagaagactgctaataaaaagcagt
mutated 8Z	451 cagctcctaagagcgaaatattgaaaagaagactgctaataaaaagcagt
4Z	491 ctgagccctctgaagaatatct
8Z	493 ctgagccctctgaagaatatct
mutated 8Z	501 ctgagccctctgaagaatatct

FIG. 16 CONT'D

16 / 16

FIG. 17

Schematic representation of the structure of pONY 8.3G +/- vector genome plasmids



SEQUENCE LISTING PART OF THE DESCRIPTION

pONY8.1Z MLVHyb (SEQ ID NO 10)

5 AGATCTTGAATAATAAAATGTGTGTTGTCCGAAATACGCGTTTTGAGATTTCTGTCCGCGACTAAATTCATGTCCGCGG
ATAGTGGTGTTTATCGCCGATAGAGATGGCGATATTGGAATAATGATATTTGAAAATATGGCATATTGAAAATGTCCG
CGATGTGAGTTTCTGTGTAACGTATATCGCCATTTTCCAAAAGTGATTTTGGGCATACGCGATATCTGGCGATAGCGC
TTATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTTGGCGGATTCTGTGTGTCGCAAAATATCCAGTTTCGA
TATAGGTGACAGCGATATGAGGCTATATCGCCGATAGAGGCGACATCAAGCTGGCACATGGCCAATGCATATCGATC
10 TATACATTGAATCAATATTGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATT
GCATACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATT
GACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCCGGTTACATAACTTACGG
TAAATGGCCCCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCC
AATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCAT
15 ATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGCCCAGTACATGACCTTACGGG
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGG
CGTGGATAGCGGTTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTGTTGGCACCAAA
ATCAACGGGACTTTCCAAAATGTCTGAACAACTGCGATCGCCCCCGCTTGACGCAATGGGCGGTAGGCGGTGACGG
TGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACGGGCGACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGT
20 GAAAAGGCCTTTGTAATAAATAAATCTCTACTCAGTCCCTGTCTAGTTTGTCTGTTTCGAGATCCTACAGTTGGCGC
CCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAACCTGGCTGATCGTAGGATCCCCGGGACAGCAGAGGA
GAACTTACAGAAAGTCTTCTGGAGGTGTTCTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCTTTGACATT
GGAGCAAGGGCTCAAGAAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAATACTGGTAACTGTAATTGGG
CGCTAAGTCTAGTAGACTTATTCATGATACCAACTTTGTAAAAGAAAAGGACTGGCAGCTGAGGGATGTCATTCCATT
25 GCTGGAAGATGTAACTCAGACGCTGTCAGGACAAGAAAGAGAGGCCTTTGAAAGAACATGGTGGGCAATTTCTGCTGT
AAAGATGGGCTCCAGATTAATAATGTAGTAGATGGAAGGCATCATTCCAGCTCCTAAGAGCGAAATATGAAAAGAA
GACTGCTAATAAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAATAAGTGGATCCCCGGGGTGCAGGAGTGGG
GAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATA
TTGGCTATTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCAT
30 OTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGC
GTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACGTCAATAATGACGTATG
TTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGCCCAG
TACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATCGGTTTTG
35 GCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAGT
TTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCTGAACAACTCCGCCCCATTGACGCAATGGGCGGTAGGC
ATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCTGGAGACGCCATCCACGCTGTTT
TGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCCCAAGCTTCAGCTGCTCGAGGATCTGCGGATCCGG
GGAATTCCCCAGTCTCAGGATCCACCATGGGGGATCCCGTGGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTAC
40 CCAACTTAATCGCCTTGACGACATCCCCCTTTCCGACGCTGGCGTAATAGCGAAGAGGCCCGACCGATCGCCCTTCC
CAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGAAAAGCTGGCTG
GAGTGCGATCTTCTGAGGCCGATACTGTCTGCTCCCTCAAACCTGGCAGATGCACGGTTACGATGCGCCCATCTACA
CCAACGTAACCTATCCATTACGGTCAATCCGCGTTTGTTCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTT
AATGTTGATGAAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTGTATGGCGTTAACTCGGCGTTTCTCTGTTG
45 GCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGGCGTCTGAATTTGACCTGAGCGCATTTTACGCGCCGG
AGAAAACCGCCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAG
CGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCATGTTGCCACTCGCTTAAATG
ATGATTTACGCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTC
TTTATGGCAGGGTGAACGCGAGGTCGCCAGCGGCACCGCGCTTTCCGGCGGTGAAATATCGATGAGCGTGGTGGTTAT

GCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCGAAATCTATCGTGCGG
TGGTTGAACTGCACACCGCCGACGGCAGCGTGATTGAAGCAGAAGCCTGCGATGTCGGTTCCGCGAGGTGCGGATTGA
AAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTGAGGCGTTAACCGTCACGAGCATCATCTCTGCATGGT
CAGGTCATGGATGAGCAGACGATGGTGCAGGATATCTGCTGATGAAGCAGAACTTTAACGCCGTGCGCTGTTCGG
5 ATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCTGTATGTGGTGGATGAAGCCAATATTGAAAC
CCACGGCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGTGCGTACCGGCGATGAGCGAACCGCTAACCGCAAT
GGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATACGA
CCGCTGTATCGTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGCGCGGAGCCGACACCACGGCC
10 AACGGCTTTGCTACCTGGAGAGACGCGCCCGTGATCCTTTGCGAATACGCCACGCGATGGGTAACAGTCTTGCGCG
TTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCGTTTACAGGGCGGCTTCGTCGGAAGTGGTGGATCAGTCGC
TGATTAATATGATGAAAACGGCAACCCGTTGTCGGCTTACGGCGGTGATTTTGCGGATACGCCAACGATCGCCAGTT
CTGTATGAACGGTCTGGTCTTTGCCGACCGACGCGCATCCAGCGTGACGGAAGCAAAACACCAGCAGAGTTTTTC
CAGTTCGGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCTGCACT
15 GGATGGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAAGGTAAACAGTTGA
TTGAACTGCCTGAACTACCGCAGCCGAGAGCGCCGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACCGCA
CCGCATGGTCAGAAGCCGGGCACATCAGCGCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCG
CCGCTCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATT
TAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAAGTCTGACGCCGCTGCGCGATCAGTTC
20 ACCCGTGACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGATTGACCCTAACGCGTGGGTGCAACGCTGG
AAGGGCGGGGCCATTACCAGGCCGAAGCAGCGTTGTGTCAGTGCACGGCAGATACACTGCTGATGCGGTGCTGATT
ACGACCGCTCAGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGAAAACCTACCGGATTGATGGTAGTGGTC
AAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATTGGCTGAACTGCCAGCTGGC
GCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAATATCCCGACCGCCTTACTGCCGCTGTTTT
25 GACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGC
CGCAATTGAATTATGGCCACACCAAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGAT
GGAAACAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGG
TGGCGAGCACTCCTGGAGCCCGTCACTATCGCGGAATTCCAGCTGAGCGCGCGTCTACCAATTACCAGTTGGTCTGG
TGTCAAAAATAATAAACCGGCGAGGGGGATCCGCGATCCGGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAG
30 TCCCCAGGCTCCCCAGCAGGCAAGATATGCAAGCATGCCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGAA
TTGGAAGAGCTTTAAATCCTGCCACATCTCATGTATCAATGCCTCAGTATGTTTAGAAAAACAAGGGGGAACTGTGGG
GTTTTATGAGGGGTTTTATAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGG
CATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCA
AACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAAC
35 AGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGC
AGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCCTATTTGAACTAACCAA
TCAGTTCGCTTCTCGCTTCTGTTGCGCGCTTCTGCTCCCCGAGCTCAATAAAGAGCCCCACAACCCCTCACTCGGGGG
CACTCAGATTCTGCGGTCTGAGTCCCTTCTGCTGGGCTGAAAAGGCCCTTTGTAATAAATAAATCTCTACTCAGTCC
CTGCTCTAGTTTGTCTGTTGAGATCCTACAGAGCTCATGCCCTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAA
40 ATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCTAATGAGTGAG
CTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCG
GCCAACCGCGCGGGAGAGGCGGTTTGGGTATTGGGCGCTTCTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTCTGT
CGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAAACGGTTATCCACAGAATCAGGGGATAACCCAGGAAAG
AACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGCGGTTTTTCCATAGGCTCCGC
45 CCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG
TTTCCCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTCTCCTTCTCG
GGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCCGGTGTAGGTGCTTTCGCTCCAAGCTGGGCTGTGT
GCACGAACCCCCGTTACGCCGACCGCTGCGCTTATCCGGTAATATCGTCTTGAGTCCAACCCGGTAAGACACGAC
TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAACAGAGCGAGGTATGTAGGCGGTGTACAGAGTCTTGAAGT

GGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAG
AGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGCAAGCAGCAGATTACGGCG
AGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACCTACGTTAAG
GGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTAAATTAAAAATGAAGTTTTAAATCAATCTAA
5 AGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCG
TTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCA
ATGATACCGGAGACCCACGCTACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGA
AGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTGCCAGTTAA
TAGTTTGGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTGGTATGGCTTCATTCAGCTCCG
10 GTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCCGATCGTT
GTCAGAAGTAAGTTGGCCGAGTGTATCACTATGGTTATGGCAGCACTGCATAATTCTTCTACTGTCATGCCATCCGT
AAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTGCC
CGGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGCTCATCTTGGAAAACGTTCTTCGGGGCG
AAAACCTCTCAAGGATCTTACCGCTGTGAGATCCAGTTCGATGTAAACCACTCGTGCACCCAACTGATCTTCAGCATCTT
15 TTACTTTACACAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGA
AATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATAT
TTGAATGTATTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCGCCGAAAAAGTGCCACCTAAATTGTAAGCGTT
AATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTAAACCAATAGGCCGAAATCGGCAAAATCCC
TTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAGAAGCGTG
20 GACTCCACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTT
TGGGGTGAGGTGCCGTAAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAAGCCAA
CCTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCGGC

pONY8.3G FB29 – (SEQ ID No 45)

25

AGATCTTGAATAATAAAATGTGTGTTGTCCGAAATACGCGTTTTGAGATTTCTGTGCC
GACTAAATTCATGTCGCGGATAGTGGTGTATTATCCCGATAGAGATGGCGATATTGGAA
AAATTGATATTTGAAAATATGGCATATTGAAAATGTCGCGGATGTGAGTTTCTGTGTAAC
TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACCGGATATCTGGCGATAGCGCT
TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGC
GCAAAATATCGAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCCCGATAGAGG
CGACATCAAGCTGGCAGATGGCAATGCATATCGATCTATACATTGAATCAATATTGGCC
ATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCA
TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
TAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCCCTGGCTGACC
GCCCAACGACCCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCC
CGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGG
ATAGCGGTTTGACTACGGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTT
GTTTTGGCACCAAAATCAACGGGACTTTCAAAATGTCGTAACAACTGCGATCGCCCCGCC
CCGTTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGT
TTAGTGAACGGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGTGAAAAGG
CCTTTGTAATAAATAAATCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTTCGAGATC
CTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAACCTGG
CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAAGTACAGAAAGTCTTCTGGAGGTGTTT

CTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCCTTTGACATTGGAGCAAGGCG
CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAACACTGGTAAGTGT
AATTGGGCGCTAAGTCTAGTAGACTTATTTTCATGATACCAACTTTGTAAGAAAAAGGAC
TGGCAGCTGAGGGATGTCATTCCATTGCTGGAAGATGTAATCAGACGCTGTCAGGACAA
GAAAGAGAGGGCTTTGAAAGAACATGGTGGGCAATTTCTGCTGTAAAGATGGGCTCCAG
ATTAATAATGTAGTAGATGGAAAGGCATCATTCCAGCTCCTAAGAGCGAAATATGAAAAAG
AAGACTGCTAATAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAACTAGTGGATC
CCCCGGGCTGAGGAGTGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCAT
TAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATA
CGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCAT
GTTGACATTGATTATTGACTAGTTATTAAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCCATATGAGGTTCCGCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGC
CCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
GGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTAC
ATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACG
TATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGAT
AGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATGACGTCAATGGGAGTTTGT
TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACTCCGCCCCATTGACGC
AAATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACC
GTCAGATCGCCTGGAGACGCCATCCACGCTGTTTGGACCTCCATAGAAGACACCGGGACC
GATCCAGCCTCCGCGCCCCAAGCTTGTGGGATCCACCGGTGCCACCATGGTGAGCAA
GGGCGAGGAGCTGTTACCGGGGTGGTGGCCATCCTGGTCGAGCTGGACGGCGACGTAAA
CGGCCACAAGTTCAGCGTGTCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC
CCTGAAGTTCATGTCACCACCGGCAAGCTGCCCGTGCCCTGGCCACCCCTCGTGACCAC
CCTGACCTACGGCGTGACGTGCTTCAGCCGCTACCCGACCATGAAAGCAGCAGCACTT
CTTCAAGTCCGCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
CGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCTGGTGAACCGCAT
CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA
CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAAGGCATCAAGGT
GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGACGCTCGCCGACCACTACCA
GCAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCAC
CCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT
CGTGACCGCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGA
CTCTAGAGTCGACCTGCAGGCATGCAAGCTTCAGCTGCTCGAGGGGGGGCCCGTACCCA
GCTTTTGTCCCTTATGAGGGTTAATTGCGCGGGAAGTATTTATCACTAATCAAGCAC
AAGTAATACATGAGAACTTTTACTACAGCAAGCACAACTCTCCAAAAATTTTGTTTT
ACAAAATCCCTGGTGAACATGATTGGAAGGGACCTACTAGGGTGCTGTGGAAGGGTGATG
GTGCAGTAGTAGTTAATGATGAAGGAAAGGGAATAATTGCTGTACCATTAAACAGGACTA
AGTTACTAATAAAACCAAAATGAGTATTGTTGCAGGAAGCAAGACCCAATACCATTGTC
AGCTGTGTTTCTGACCTCAATATTTGTTATAAGGTTTGATATGAATCCAGGGGGAATC
TCAACCCCTATTACCAACAGTCAGAAAAATCTAAGTGTGAGGAGAACACAATGTTTCAA
CCTTATTGTTATAATAATGACAGTAAGAACAGCATGGCAGAATCGAAGGAAGCAAGAGAC
CAAGAATGAACCTGAAAGAAAGATCTAAAGAAAGAAAAAGAAATGACTGGTGGAAAA
TAGGTATGTTTCTGTTATGCTTAGCAGGAACACTGAGGAGAACTTTTGGTGGTATGAAG
GACTCCACAGCAACATTATATAGGGTTGGTGGCGATAGGGGGAAGATTAAACGGATCTG
GCCAATCAAATGCTATAGAATGCTGGGGTTCCCTCCCGGGGTGTAGACCATTTCAAAAT
ACTTCAGTTATGAGACCAATAGAAGCATGCATATGGATAATAATACTGCTACATTATTAG
AAGCTTTAACCAATATAACTGCTCTATAAATAACAAAACAGAATTAGAAACATGGAAGTT

AGTAAAGACTTCTGGCATAACTCCTTTACCTATTTCTTCTGAAGCTAACACTGGACTAAT
TAGACATAAGAGAGATTTTGGTATAAGTGCAATAGTGGCAGCTATTGTAGCCGCTACTGC
TATTGCTGCTAGCGCTACTATGTCTTATGTTGCTCTAACTGAGGTAAACAAAATAATGGA
AGTACAAAATCATACTTTTGAGGTAGAAAATAGTACTCTAAATGGTATGGATTTAATAGA
ACGACAAAATAAGATATTATATGCTATGATTCTTCAAACACATGCAGATGTTCAACTGTT
AAAGGAAAGACAACAGGTAGAGGAGACATTTAATTTAATTGGATGTATAGAAAGAACACA
TGTAATTTTGTCACTGGTCATCCCTGGAATATGTCATGGGGACATTTAAATGAGTCAAC
ACAATGGGATGACTGGGTAAAGCAAAATGGAAGATTTAAATCAAGAGATACTAACTACACT
TCATGGAGCCAGGAACAATTTGGCACAATCCATGATAACATTCAATACACCAGATAGTAT
AGCTCAATTTGGAAAAAGACCTTTGGAGTCATATTGGAAATTGGATTCTGGATTGGGAGC
TTCCATTATAAAATATATAGTGATGTTTTGCTTATTTATTGTTACTAACCTCTTCGCC
TAAGATCCTCAGGGCCCTCTGGAAGGTGACCAGTGGTGCAGGGTCTCCGGCAGTCGTTA
CCTGAAGAAAAAATTCATCACAAACATGCATCGCGAGAAGACACCTGGGACCAGGCCCA
ACACAACATACACCTAGCAGGCGTGACCGTGGATCAGGGGACAAATACTACAAGCAGAA
GTACTCCAGGAACGACTGGAATGGAGAATCAGAGGAGTACAACAGGCGGCCAAAGAGCTG
GGTGAAGTCAATCGAGGCATTTGGAGAGAGCTATATTTCCGAGAAGACCAAAGGGGAGAT
TTCTCAGCCTGGGGCGGCTATCAACGAGCACAGAAGCGCTCTGGGGGGAACAATCCTCA
CCAAGGGTCTTAGACCTGGAGATTCGAAGCGAAGGAGGAAACATTTATGACTGTTGCAT
TAAAGCCCAAGAAGGAACTCTCGCTATCCCTTGCTGTGGATTCCCTTATGGCTATTTG
GGGACTAGTAATTATAGTAGGACGCATAGCAGGCTATGGATTACGTGGACTCGCTGTTAT
AATAAGGATTTGTATTAGAGGCTTAAATTTGATATTTGAAATAATCAGAAAAATGCTTGA
TTATATTGGAAGAGCTTAAATCCTGGCACATCTCATGTATCAATGCCTCAGTATGTTA
GAAAAACAAGGGGGGAACTGTGGGGTTTTATGAGGGGTTTTATAATGATTATAAGAGT
AAAAAGAAAGTTGCTGATGCTCTCATAACCTTGATAACCCAAAGGACTAGCTCATGTTG
CTAGGCAACTAAACCGCAATAACCGCATTTGTGACCGAGTCCCCATTTGGTGACGCGTT
AACTCTCTGTTTTACAGTATATAAGTGCTTGATTTCTGACAATTGGGCACTCAGATTCT
GCGGTCTGAGTCCCTCTCTGCTGGGCTGAAAAGGCCTTTGTAATAAATAAATCTCTA
CTCAGTCCCTGTCTCTAGTTTGTCTGTTGAGATCCTACAGAGCTCATGCCTTGGCGTAA
TCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATCCACACAACATA
CGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTA
ATTGCGTTGCGCTCACTGCCCCGTTTCCAGTCGGGAAACCTGTCGTGCCAGTGATGCCCG
GGCGGCGGAGGCGGCTACGTGAACCATCACCCAAATCAAGTTTTTTGCGGTCGAGGTGC
CGTAAAGCTCTAAATCGGAACCCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAG
CCGGCGAAGCTGGCGAGAAAGGAAGGGAAGGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTG
GCAAGTGTAAGCGGTACGCTGCGCGTAACCAACACACCCGCGCGCTTAATGCGCCGCTA
CAGGGCGCGTCCATTGCGCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGG
CCTCTTCGCTATTACGCCAGCCCGGATCGATCCTTATCGGATTTTACCACATTTGTAGAG
GTTTTACTTGCTTTAAAAAACCTCCACATCTCCCCCTGAACCTGAAACATAAAATGAAT
GCAATTGTTGTTGTTAACTTGTTTATTGACGCTTATAATGGTTACAAATAAAGCAATAGC
ATCACAAATTTACAAATAAAGCATTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAA
CTCATCAATGTATCTTATCATGTCTGCTCGAAGCATTAAACCCTCACTAAAGGGAAGCGGC
CGCCCCGGTGCAGTTCACAGGTGTTTGGCGGCTCTTTGGAGTCTCCGGGCTCAAGACG
CGGGGGCTGCTCTGCTCGCCCCACAGCCTTTCTTGCCCTCTGGTAGCCTCCCATGCG
GAGAAATCGCCCTCTGGTCTCGCGGAAGTAGAGCTCCCTCCAGATGCCGCGATTACC
TCTCCAGCTCTTTAGCGGCTTGTGTCACGCCCCCTAATTCTCCATTCCAGCCTTTCTTGG
AGGACCTCGGCTTGCAAAATCTGGCCCCTAATCCACCTATCCCTCTGGAGGGTGTGTGC
TGGGTGGGACCGGGGCGAGGTGTCTTCTGGCGATGCAGGTCTGGCTAGGAATCTTCTCC
TCGGGCAGGACTGTCTCAGCACGCGGCACCACTGGTCCCCCTCCAGGGGGCCTGTGGG
TCGATCTTCCACCAGTCGTTGCGGCGCTTCTCCTTTGCTCTTCTTGGAGGTTTCATC

TCTTGATCCCTGGCCTCCTTGCTCTCAGCCATGGTGGCGAATTCTCGAGGCTAGCCTCCC
GGTGGTGGGTGCGTGGTCCCTGGGCAGGGGTCTCCAGATCCCGGACGAGCCCCAAATGA
AAGACCCCGAGACGGGTAGTCAATCACTCTGAGGAGACCCTCCCAAGGAACAGCGAGAC
CACGAGTCGGATGCAACAGCAAGAGGATTTATTGGATACACGGGTACCCGGGCGACTCAG
TCTATCGGAGGACTGGCGCGCCGAGTGAGGGGTTGTGAGCTCTTTATAGAGCTCGGGAA
GCAGAAGCGCGCAACAGAAAGCGAGAAAGCAGGCTGATTGGTTAATTCAAATAAGGCACAG
GGTCATTTCAAGTCTTGGGGAGCCTGGAAACATCTGATGGGTCTTAAGAAACTGCTGA
GGGTTGGGCCATATCTGGGGACCATCTGTTCTTGGCCCCGGCGGGGCCGAACCGCGGT
GACCATCTGTTCTTGGCCCCGGCGGGGCCGAAACTGCTCACCAGATATCCTGTTTG
GCCAACGTTAGCTGTTTTCTGTGTACCCGCCCTTGATCTGAACCTCTCTATTCTTGGTTT
GGTATTTTCCATGCCTTGCAAAATGGCGTACTGCGGCTATCAGGCTAAGCAATTTGAG
ATCTGGCCGAGGCGGCTACTCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGT
TTGCGTATTGGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTGCTCGG
CTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGG
GATAACGCAGGAAAGAACATGTATAACTTCGTATAATGTATGCTATACGAAGTTATACAT
GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTT
CCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG
AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC
TCTGTTCGACCCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGT
GGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTCGCTCCAA
GCTGGGCTGTGTGACGAACCCCGTTACGCCGACCGCTGCGCCTTATCCGTAACATA
TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA
CAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAA
CTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACCAACCGCTGGTAGCGGTGGTTT
TTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT
CTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCAT
GAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAAAAATGAAGTTTAAATC
AATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGC
ACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA
GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA
CCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCG
CAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGC
TAGAGTAAGTAGTTCCGCGAGTTAATAGTTTGGCAACGTTGTTGCCATTGCTACAGGCAT
CGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTAGCTCCGTTCCCAACGATCAAG
GCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGTCTCTCGGTCCTCCGAT
CGTTGTGAGAAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAA
TTCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAAGTACTCAACCAA
GTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGA
TAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATTTGGAACGTTCTTTCGGG
GCGAAAACTCTAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGC
ACCCAACTGATCTTCAGCATCTTTACTTTACACAGCGTTTCTGGGTGAGCAAAAACAGG
AAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACT
CTTCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACAT
ATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAAGT
GCCACCTAAATTGTAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTGTAAATC
AGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAG
ACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAGAAGCGTG
GACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGATAAC

TTCGTATAATGTATGCTATACGAAAGTTATCACTACGTGAACCATCACCTAATCAAGTTT
TTTGGGGTCGAGGTGCCGTAAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTAG
AGCTTGACGGGAAAGCCAACCTGGCTTATCGAAATTAATACGACTCACTATAGGGAGAC
CGGC

pONY8.3G FB29 + (SEQ ID No 46)

AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACCGTTTTGAGATTCTGTGCGC
GACTAAATTCATGTGCGCGGATAGTGGTGTATCGCCGATAGAGATGGCGATATTGGAA
AAATTGATATTTGAAAATATGGCATATTGAAAATGTGCGCGATGTGAGTTTCTGTGTAA
TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACGCGATATCTGGCGATAGCGCT
TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGTC
GCAAATATCCGAGTTTCGATATAGGTGACAGCGATATGAGGCTATATCGCCGATAGAGG
CGACATCAAGCTGGCACATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
ATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCA
TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
TAGCCCATATATGGAGTTCGCGTTACATAAATTACGGTAAATGGCCCGCTGCGTGACC
GCCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCC
CGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGG
ATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTT
GTTTTGGCACCAAAATCAACGGGACTTTCAAAATGTGTAACAACCTGCGATCGCCCGCC
CCGTTGACGCAAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGCTCGT
T TAGTGAACCGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGG
CCTTTGTAATAATATAATTCTCTACTCAGTCCCTGTCTCTAGTTGTCTGTTGAGATC
CTACAGTTGGCGCCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAACCTGG
CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAATTACAGAAGTCTTCTGGAGGTGTT
CTGGCCAGAACACAGGAGGACAGGTAAAGATTGGGAGACCCTTTGACATTGGAGCAAGGCG
CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAATACTACTGGTAACTGT
AATTGGGCGCTAAGTCTAGTAGACTTATTTATGATACCAACTTTGTAAAAGAAAAGGAC
TGGCAGCTGAGGGATGTCAATTGCTGGAAGATGTAATCAGACGCTGTGAGGACAA
GAAAGAGAGGCCCTTTGAAAGAACATGGTGGGCAATTTCTGCTGTAAAGATGGGCCTCCAG
ATTAATAATGTAGTAGATGGAAAGGCATCATTCCAGCTCCTAAGAGCGAAATATGAAAAG
AAGACTGCTAATAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAACTAGTGGATC
CCCCGGGCTGCAGGAGTGGGGAGGCACGATGGCCGCTTTGGTGGAGGCGGATCCGGCCAT
TAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATA
CGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCAT
GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCCATATATGGAGTTCGCGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACCGC
CCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
GGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTAC
ATCAAGTGATCATATGCCAAGTACGCCCTTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACG
TATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGAT
AGCGGTTTGACTACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTTGT
TTTGGCACCAAAATCAACGGGACTTTCAAAATGTGTAACAACCTCCGCCCATTTGACGC

AAATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACC
GTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACC
GATCCAGCCTCCGCGGCCCCAAGCTTGTGGGATCCACCGGTCCGCCACCATGGTGAGCAA
GGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAA
CGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC
CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTGGCCCCACCTCGTGACCAC
CCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGCACTT
CTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTCAAGGACGA
CGGCAACTACAAGACCCCGCGCGAGGTGAAGTTCGAGGGCGACACCTGGTGAACCGCAT
CGAGCTAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA
CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGT
GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCA
GCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCAC
CCAGTCCGCCCTGAGCAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTT
CGTGACCGCCCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGA
CTCTAGAGTCGACCTGCAGGCATGCAAGCTTCACTGCTCGAGGGGGGGCCCGGTACCCA
GCTTTTGTTCCTTTAGTGAGGGTTAATTGCGCGGAAGTATTTATCACTAATCAAGCAC
AAGTAATACATGAGAACTTTTACTACAGCAAGCACAATCCTCCAAAAAATTTGTTTT
ACAAAATCCCTGGTGAACATGATTGGAAGGGACCTACTAGGGTCTGTGGAAGGGTGATG
GTGCAGTAGTAGTAAATGATGAAGGAAAGGAATAATTGCTGTACCATTAACAGGACTA
AGTTACTAATAAAACCAATTGAGTATTGTTGCAGGAAGCAAGACCCAACTACCAATTGTC
AGCTGTGTTTCTGACCTCAATATTTGTTATAAGGTTTGATATGAATCCAGGGGGAATC
TCAACCCCTATTACCCAACAGTCAGAAAAATCTAAGTGTGAGGAGAACAATGTTTCAA
CCTTATTGTTATAATAATGACAGTAAGAACAGCATGGCAGAATCGAAGGAAGCAAGAGAC
CAAGAAATGAACCTGAAAGAAGAAATCTAAAGAAGAAAAAAGAAGAAATGACTGGTGAAAA
TAGGTATGTTTCTGTTATGCTTAGCAGGAACCTACTGGAGGAATACTTTGGTGGTATGAAG
GACTCCACAGCAACATTATATAGGGTTGGTGGCGATAGGGGGAAGATTAAACGATCTG
GCCAATCAAATGCTATAGAATGCTGGGGTTCTTCCCGGGGTGTAGACCATTCAAAT
ACTTCAGTTATGAGACCAATAGAAGCATGCATATGGATAATAATACTGCTACATTATTAG
AAGCTTTAACCAATATACTGCTCTATAAATAACAAAACAGAATTAGAAACATGGAAGTT
AGTAAAGACTTCTGGCATAACTCCTTTACCTATTTCTTCTGAAGCTAACACTGGACTAAT
TAGACATAAGAGAGATTTTGGTATAAGTGCAATAGTGGCAGCTATTGTAGCCGCTACTGC
TATTGCTGCTAGCGCTACTATGTCTTATGTTGCTCTAAGTGGTTAACAAAATAATGGA
AGTACAAAATCATACTTTTGGAGTAGAAAATAGTACTCTAAATGGTATGGATTAAATAGA
ACGACAAAATAAGATATTATATGCTATGATTCTTCAAACACATGCAGATGTTCAACTGTT
AAAGGAAAGACAACAGGTAGAGGAGACATTTAATTTAATTGGATGTATAGAAAGAACACA
TGTATTTTGTACATACTGGTCAATCCCTGGAATATGTCATGGGGACATTTAAATGAGTCAAC
ACAATGGGATGACTGGGTAAGCAAAATGGAAGATTTAAATCAAGAGATACTAACTACACT
TCATGGAGCCAGGAACAATTTGGCACAATCCATGATAACATTCAATACACCAGATAGTAT
AGCTCAATTTGGAAAAGACCTTTGGAGTCATATTGGAATTTGGATTCTGGATTGGGAGC
TTCCATTATAAAATATATAGTGATGTTTTTGTCTATTTATTTGTTACTAACCTCTTCGCC
TAAGATCCTCAGGGCCCTCTGGAAGGTGACCAAGTGGTGCAGGGTCTCCGGCAGTCGTTA
CCTGAAGAAAAAATCCATCACAACATGCATCGCGAGAAGACACCTGGGACCAGGCCCA
ACACAACATACACCTAGCAGGCGTGACCGGTGGATCAGGGGACAAATACTACAAGCAGAA
GTAATCCAGGAACGACTGGAATGGAGAATCAGAGGAGTACAACAGGGCGGCAAGAGCTG
GGTGAAGTCAATCGAGGCATTTGGAGAGAGCTATATTTCCGAGAAGACCAAGGGGAGAT
TTCTCAGCCTGGGGCGGCTATCAACGAGCACAAGAACGGCTCTGGGGGGAACAATCCTCA
CCAAGGGTCTTAGACCTGGAGATTGGAAGCGAAGGAGGAAACATTTATGACTGTTGCAT
TAAAGCCCAAGAAGAACTCTCGCTATCCCTTGTGTGGATTTCCTTATGGCTATTTTG

GGGACTAGTAATTATAGTAGGACGCATAGCAGGCTATGGATTACGTGGACTCGCTGTTAT
AATAAGGATTTGTATTAGAGGCTTAAATTTGATATTTGAAATAATCAGAAAAATGCTTGA
TTATATTGGAAGAGCTTTAAATCCTGGCACATCTCATGTATCAATGCCTCAGTATGTTTA
GAAAAACAAGGGGGAACTGTGGGGTTTTATGAGGGGTTTTATAAATGATTATAAGAGT
AAAAAGAAAGTTGCTGATGCTCTCATAACCTTGATAACCCAAAGGACTAGCTCATGTTG
CTAGGCAACTAAACCGCAATAACCGCATTGTGACGCGAGTTCCCCATTGGTGACGCGTT
AACTTCCTGTTTTACAGTATATAAGTGCTTGATTTCTGACAATTGGGCACTCAGATTCT
GCGGTCTGAGTCCCTTCTGCTGGGCTGAAAAGGCCTTTGTAATAAATATAATTCTCTA
CTCAGTCCCTGTCTCTAGTTTGTCTGTTTCGAGATCCTACAGAGCTCATGCCTTGGCGTAA
TCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATCCACACAACATA
CGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCTAATGAGTGAGCTAACTCACATTA
ATTGCGTTGGGCTCACTGCCGCTTCCAGTCGGGAAACCTGCTGCCAGAGTAGGCCG
CCTCGGCCAGATCTCAAATGCTTAGCCTGATAGCCGAGTAACGCCATTTTGCAAGGCA
TGGAATAATACCAACCAAGAATAGAGAAGTTCAGATCAAGGGCGGGTACACGAAAACAG
CTAAGCTTGGGCCAAACAGGATATCTGCGGTGAGCAGTTTCGGCCCCGGCCGGGGCCAA
GAACAGATGGTCACCGCGTTTCGGCCCCGGCCGGGGCCAAAGACAGATGGTCCCCAGAT
ATGGCCCAACCTCAGCAGTTTCTTAAGACCCATCAGATGTTCCAGGCTCCCCAAGGA
CCTGAAATGACCTGTGCCTATTGTAATTAACCAATCAGCCTGCTTCTCGCTTCTGTTT
GCGCGCTTCTGCTTCCCGAGCTCTATAAAAGAGCTCACAACCCCTCACTCGGCGCGCCAG
TCCTCCGATAGACTGAGTCGCGCGGTACCCGTGTATCCAATAAATCCTTCTGCTGTTGC
ATCCGACTCGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCAGAGTGATTGACTACCCGT
CTCGGGGGTCTTTCATTGGGGGCTCGTCCGGGATCTGGAGACCCCTGCCAGGGACCAC
CGACCCACCACCGGGAGGCTAGCCTCGAGAATTCGCCACCATGGCTGAGAGCAAGGAGGC
CAGGGATCAAGAGATGAACCTCAAGGAAGAGAGCAAAGAGGAGAAGCGCCGCAACGACTG
GTGGAAGATCGACCCACAAGGCCCTGGAGGGGGACCAAGTGGTGGCGGTGCTGAGACA
GTCCCTGCCGAGGAGAAGATTCTAGCCAGACCTGCATCGCCAGAAGACACCTCGGCC
CGGTCCCAACCCAGCACACCCCTCCAGAAGGGATAGGTGGATTAGGGGCCAGATTTTGCA
AGCCGAGGTCTCCAAGAAAGGCTGGAATGGAGAATTAGGGGCGTGCAACAAGCCGCTAA
AGAGCTGGGAGAGGTGAATCGCGCATCTGGAGGGAGCTCTACTCCGCGAGGACCAGAG
GGGCGATTTCTCCGCATGGGGAGGCTACCAGAGGGCACAAGAAAGGCTGTGGGGCGAGCA
GAGCAGCCCCCGCGTCTTGAAGCCCGGAGACTCCAAAAGACGCCGCAAAACACCTGTGAAG
TCGACCCGGGCGGCCCTTCCCTTTAGTGAGGGTTAATGCTTCGAGCAGACATGATAAGA
TACATTGATGAGTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTGT
GAAATTTGTGATGCTATTGCTTTATTTGTAACCAATTATAAGCTGCAATAAACAAGTTAAC
AACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGATGTGGGAGGTTTTTAA
AGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGATCGATCCGGGCTGGCGTA
ATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAAT
GGACGCGCCTGTAGCGCGCATTAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGAC
CGCTACACTTGGCAGCGCCCTAGCGCCCGCTCCTTTCGCTTCTTCCCTTCTTCTCGC
CACGTTTCGCCGCTTTCCTCGTCAAGCTCTAAATCGGGGGCTCCCTTAGGGTTCCGATT
TAGAGCTTTACGGCACCTCGACCGCAAAAACTTGATTGGGTGATGGTTCACGTAGGCC
GCCTCGGCCGCCGGGCATCACTGCAATTAATGAATCGGCCAACGCGGGGAGAGGCGGT
TTGCGTATTGGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTCGCTCGGTCTGTTCCG
CTGGCGGAGCGGTATCAGTCACTCAAAGGCGGTAAATACGGTTATCCACAGAATCAGGG
GATAACGCAGGAAAGAACATGTATAACTTCGTATAATGTATGCTATACGAAGTTATACAT
GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGCTGGCGTTTT
CCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAGAGGTGGCG
AAACCCGACAGGACTATAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGGCGCT
TCCTGTTCCGACCTGCGCTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGT

GGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCAA
GCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTA
TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA
CAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAA
CTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCCGCTGGTACGGGTGGTTT
TTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT
CTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACTCACGTTAAGGGATTTTGGTCAT
GAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTTTAAATC
AATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGC
ACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCTGACTCCCCGTCGTGTA
GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGA
CCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCG
CAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC
TAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGGCAACGTTGTTGCCATTGCTACAGGCAT
CGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGTCCGGTCCCAACGATCAAG
GCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCCGAT
CGTTGTGAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAA
TTCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA
GTCACTTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGA
TAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGG
GCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGC
ACCCAACCTGATCTTCAGCATCTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGG
AAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACT
CTTCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACAT
ATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAAGT
GCCACCTAAATGTAGCGTTAATAATTTTGTAAAAATTCGCGTTAAATTTTTGTAAATC
AGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAG
ACCGAGATAGGGTTGAGTGTGTTCCAGTTTGAACAAGAGTCCACTATTAAGAAGCGTG
GACTCCAAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGATAAC
TTCGTATAATGTATGTATACGAAGTTATCACTACGTGAACCATCACCTAATCAAGTTT
TTTGGGGTTCGAGGTGCCGTAAAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTAG
AGCTTGACGGGGAAGCCAACCTGGCTTATCGAAATTAATACGACTCACTATAGGGAGAC
CGGC

pONY8.3GPGK – (SEQ ID No 47)

AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACGCGTTTGTGAGATTTCTGTGCC
GACTAAATTCATGTCCGCGATAGTGGTGTATTCGCCGATAGAGATGGCGATATTGGAA
AAATTGATATTTGAAAAATATGGCATATTGAAAATGTCCCGATGTGAGTTTCTGTGTAAC
TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACCGGATATCTGGCGATAGCGCT
TATATCGTTTACGGGGATGGCGATAGACGACTTGGTGACTTGGGCGATTCTGTGTGTC
GCAAAATATCGAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCCGATAGAGG
CGACATCAAGCTGGCACATGGCCAAATGCATATCGATCTATACATTGAATCAATATTGGCC
ATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCA
TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
TAGCCCATATATGGAGTTCGCGTTACATAACTACGGTAAATGGCCCGCTGGCTGACC
GCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT

AGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGT
ACATCAAGTGATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCC
CGCCTGGCAATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGG
ATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTT
GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTGCGATCGCCCGCC
CCGTTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGT
TTAGTGAACCGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGG
CCTTTGTAATAAATATAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTGAGATC
CTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAACCTGG
CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAACTTACAGAAGTCTTCTGGAGGTGTTT
CTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCTTTGACATTGGAGCAAGGCG
CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAAGTCTGTTAACTGT
AATTGGGCGCTAAGTCTAGTAGACTTATTTATGATACCAACTTTGTAAGGAAAAGGAC
TGGCAGCTGAGGGATGTCTATTCCATTGCTGGAAGATGTAAGTACAGACGCTGTGAGGACA
GAAAGAGAGGCGCTTTGAAAGAACATGGTGGGCAATTCTGCTGTAAGATGGGCGCTCCAG
ATTAATAATGTAGTAGATGGAAGGCATCATTCAGCTCCTAAGAGCGAAATATGAAAAG
AAGACTGCTAATAAAAGCAGTCTGAGCCCTCTGAAGAATATCTAGAACTAGTGGATC
CCCCGGGCTGCAGGAGTGGGAGGACGATGCGCGCTTTGGTCGAGGCGGATCCGGCCAT
TAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATA
CGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCAT
GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGCTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGC
CCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
GGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTAC
ATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACG
TATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGAT
AGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGT
TTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGC
AAATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACC
GTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACC
GATCCAGCCTCCGCGGCCCAAGCTTGTGGATCCACCGGTGCCACCATGGTGAGCAA
GGGCGAGGAGCTGTTACCGGGGTGGTGGCCATCCTGGTCGAGCTGGACGGCGACGTAAA
CGGCCACAAGTTACGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC
CCTGAAGTTATCTGCACCACGGCAAGCTGCCCGTGCCCTGGCCACCCTCGTGACCAC
CCTGACCTACGGCGTGACGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGCACTT
CTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
CGGCAACTACAAGACCCGCGCGAGGTGAAGTTGAGGGCGACACCTGGTGAACCGCAT
CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA
CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAAGGCATCAAGGT
GAAGTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCA
GCAGAACACCCCATCGGGCAGGGCCCCGTGTGCTGCCCCGACAACCACTACCTGAGCAC
CCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCATGGTCTGCTGGAGTT
CGTGACCGCCGCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCGCGA
CTCTAGAGTCGACCTGCAGGCATGCAAGCTTCAGTGTCTGAGGGGGGGCCCGGTACCCA
GCTTTTGTCCCTTTAGTGAGGGTTAATTGCGCGGGAAGTATTTATCACTAATCAAGCAC
AAGTAATACATGAGAACTTTTACTACAGCAAGCACAATCCTCCAAAAATTTTGTTTT
ACAAAATCCCTGGTGACATGATTGGAAGGGACCTACTAGGGTGTGTGGAAGGGTGATG

GTGCAGTAGTAGTTAATGATGAAGGAAAGGGAATAATTGCTGTACCATTAACCAGGACTA
AGTTACTAATAAAACCAAATTGAGTATTGTTGCAGGAAGCAAGACCCAACTACCATTGTC
AGCTGTGTTTCTGACCTCAATATTTGTTATAAGGTTTGATATGAATCCCAGGGGGAATC
TCAACCCCTATTACCCAACAGTCAGAAAAATCTAAGTGTGAGGAGAACACAATGTTTCAA
CCTTATTGTTATAATATGACAGTAAGAACAGCATGGCAGAATCGAAGGAAGCAAGAGAC
CAAGAATGAACCTGAAAGAAGAATCTAAAGAAGAAAAAGAAGAAATGACTGGTGAAAAA
TAGGTATGTTTCTGTTATGCTTAGCAGGAACACTGGAGGAATACTTTGGTGGTATGAAG
GACTCCACAGCAACATTATATAGGGTTGGTGGCGATAGGGGGAAGATTAAACGGATCTG
GCCAATCAAATGCTATAGAATGCTGGGGTTCCTCCCGGGGTGTAGACCATTTCAAAATT
ACTTCAGTTATGAGACCAATAGAAGCATGCATATGGATAATAACTGCTACATTATTAG
AAGCTTTAACCAATATAACTGCTCTATAAATAACAAAACAGAATTAGAAACATGGAAGTT
AGTAAAGACTTCTGGCATAACTCCTTTACCTATTTCTTCTGAAGCTAACACTGGACTAAT
TAGACATAAGAGAGATTTTGGTATAAGTGCAATAGTGGCAGCTATTGTAGCCGCTACTGC
TATTGCTGCTAGCGCTACTATGCTTATGTTGCTCTAAGTGGTTAACAAAATAATGGA
AGTACAAAATCATACTTTTGAGGTAGAAAATAGTACTCTAAATGGTATGGATTATAAGA
ACGACAAAATAAGATATTATATGCTATGATTCTTCAAACACATGCAGATGTTCAACTGTT
AAAGGAAAGACAACAGGTAGAGGAGACATTTAATTTAATTGGATGTATAGAAAGAACACA
TGTATTTTGTCTACTGGTCACTCCCTGGAATATGTCATGGGGACATTTAATGAGTCAAC
ACAAATGGGATGACTGGGTAAAGCAAAATGGAAGATTTAAATCAAGAGATACTAACTACACT
TCATGGAGCCAGGAACAATTTGGCACAATCCATGATAACATTCAATACACCAGATAGTAT
AGCTCAATTTGGAAAAGACCTTTGGAGTCAATTTGGAATTTGGATTCTGGATTGGGAGC
TTCCATTATAAAATATATAGTGATGTTTTTGTCTATTTATTTGTTACTAACCTCTTCGCC
TAAGATCCTCAGGGCCCTCTGGAAGGTGACCAGTGGTGCAGGGTCTCCGGCAGTCGTTA
CCTGAAGAAAAAATTCATCACAACATGCATCGCGAGAAGACACCTGGGACCAGGCCCA
ACACAACATACACCTAGCAGGCGGTGACCGGTGGATCAGGGGACAAATACTACAAGCAGAA
GTACTCCAGGAACGACTGGAATGGAGAATCAGAGGAGTACAACAGCGGCCAAAGAGCTG
GGTGAAGTCAATCGAGGCATTTGGAGAGAGCTATATTTCCGAGAAGACCAAAGGGGAGAT
TTCTCAGCCTGGGGCGGCTATCAACGAGCACAAGAACGGCTCTGGGGGGAACAATCCTCA
CCAAGGGTCTTAGACCTGGAGATTGGAAGCGAAGGAGGAAACATTTATGACTGTTGCAT
TAAAGCCCAAGAAGGAACTCTCGCTATCCCTTGCTGTGGATTTCCTTATGGCTATTTTG
GGGACTAGTAATTATAGTAGGACGCATAGCAGGCTATGGATTACGTGGACTCGCTGTTAT
AATAAGGATTTGTATTAGAGGCTTAAATTTGATATTTGAAATAATCAGAAAAATGCTTGA
TTATATTGGAAGAGCTTTAAATCCTGGCACATCTCATGTATCAATGCCTCAGTATGTTTA
GAAAAACAAGGGGGAACTGTGGGGTTTTATGAGGGGTTTTATAAATGATTATAAGAGT
AAAAAGAAAGTTGCTGATGCTCTCATAACCTTGATAACCCAAAGGACTAGCTCATGTTG
CTAGGCAACTAAACCGCAATAACCGCATTTGTGACGCGAGTTCCTCATTTGGTGACGCGTT
AACTTCCTGTTTTACAGTATATAAGTGCTTGTATTCTGACAATTGGGCACTCAGATTCT
GCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGGCCTTTGTAATAAATATAATTCTCTA
CTCAGTCCCTGTCTAGTTTGTCTGTTTCGAGATCTACAGAGCTCATGCCTTGGCGTAA
TCATGGTCATAGCTGTTTCTGTGTGAAATTTGTTATCCGCTCACAATCCACACAACATA
CGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCCTAATGAGTGAGCTAACTCACATTA
ATTGCGTTGGCGTCACTGCCCCGTTTCCAGTCGGGAAACCTGTCTGCCAGTGATGCCCG
GGCGGCGGAGGCGGCCTACGTGAACCATCACCCAAATCAAGTTTTTTGCGGTGAGGTGC
CGTAAAGCTCTAAATCGGAACCTAAAGGGAGCCCCGATTAGAGCTTGACGGGGAAG
CCGGCGAACGTGGCGAGAAAGGAAGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTG
GCAAGTGTAGCGGTACGCTGCGCGTAACCAACACACCCGCGCGCTTAATGCGCCGCTA
CAGGGCGCGTCCATTCCGCTTACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGG
CCTCTTCGCTATTACGCCAGCCGGATCGATCCTTATCGGATTTTACCACATTTGTAGAG
GTTTTACTTGCTTTAAAAAACCTCCACATCTCCCCCTGAACCTGAAACATAAAATGAAT

GCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAGCAATAGC
ATCACAAATTTACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGCCAAA
CTCATCAATGTATCTTATCATGTCTGCTCGAAGCATTAAACCTCACTAAAGGGAAGCGGC
CGCCCCGGTCTGACTTCACAGGTGTTTGCGGCGTCTTTTGGAGTCTCCGGGCTCAAGACG
CGGGGGCTGCTCTGCTCGCCCCACAGCCTTCTTGTCCTCTGGTAGCCTCCCCATGCG
GAGAAATCGCCCTCTGCTCTCGCGGAAGTAGAGTCCCTCCAGATGCCGCGATTACCC
TCTCCAGCTCTTTAGCGGCTTGTTGCACGCCCTAATTCTCCATTCCAGCCTTCTTG
AGGACCTCGGCTTGCAAAATCTGGCCCTAATCCACCTATCCCTTCTGGAGGGTGTGTGC
TGGGTGGGACCGGGGCGAGGTGCTTCTGGCGATGCAGGTCTGGTAGGAATCTTCTCC
TCGGGCAGGACTGTCTCAGCACGCGGCACTGGTCCCCCTCCAGGGGGCTTGTGGG
TCGATCTTCCACCAGTCGTTGCGGCGCTTCTCTCTTTGCTCTTCTTGTAGGTTTATC
TCTTGATCCCTGGCCTCTTGCTCTCAGCCATGGTGGCGAATTCTCGAGGCTAGCCTGGG
GAGAGAGGTGGTGATTCGGTCAACGAGGGAGCCGACTGCCGACGTGCGCTCCGGAGGGT
TGCAGAAATGCGGAACACCGCGCGGCGAGGAACAGGGCCCACTACCGCCCCACACCCCG
CCTCCCGACCGCCCTTCCCGGCGCTGCTCTCGGCGCGCCCCGCTGAGCAGCGCTAT
TGGCCACAGCCATCGCGGTGCGGCGCTGCCATTGCTCCCTGGCGCTGTCCGTCTGCGA
GGGTACTAGTGAGACGTGCGGCTTCCGTTTGTACGTCCGGCACGCGCGAACCAGCAAGG
AACCTTCCCGACTTAGGGGCGGAGCAGGAAGCGTCGCCGGGGGGCCACAAGGGTAGCGG
CGAAGATCCGGGTGACGTGCGAACGGACGTGAAGAAATGTGCGAGACCCAGGGTCTGGCG
CGCTGCGTTTCCCGGAACACCGCCAGAGCAGCGCGTCCCTGCGCAAAACCCAGGGCTGC
CTTGAAAAAGGCGCAACCCCAACCCAGATCTGGCCGAGGCGGCTACTCTGCATTAATG
AATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGGCGCTTCCGCTTCTCGCT
CACTGACTCGCTGCGCTCGGTGCTTGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGC
GGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTATAACTTCGT
ATAATGTATGCTATACGAAGTTATACATGTGAGCAAAAAGGCCAGCAAAAGGCCAGGAACC
GTAAAAAGGCGCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATACA
AAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGT
TTCCCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCCGACCCCTGCCGCTTACCGGATACC
TGTCGCGCTTCTCCCTTCCGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATC
TCAGTTCCGGTGTAGGTGCTTCCGCTCAAGCTGGGCTGTGTGACGAACCCCCGTTACG
CCGACCGCTGCGCCTTATCCGGTAATATCGTCTTGAGTCAACCCGGTAAGACACGACT
TATCGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTG
CTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGGACAGTATTGGTA
TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCA
AACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAA
AAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACG
AAAATCAGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCC
TTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATAGTAAACTTGGTCTG
ACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTTAT
CCATAGTTGCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTG
GCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTACCGGCTCCAGATTTATCAGCAA
TAAACCAGCCAGCCGAAGGGCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCTCCA
TCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCCGCAAGTTAAGTTTGC
GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCOTCGTTTGGTATGGCTT
CATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAA
AAGCGGTTAGTCTCTTCCGCTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTAT
CACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCTATGCCATCCGTAAGATGCT
TTTCTGTGACTGGTAGTACTCAACCAAGTCATTCTGAGAATAAGTGTATGCGGCGACCGA
GTTGCTCTTGGCGGCTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAG

TGCTCATCATTGGAACGTTCTTCGGGGCGAAACTCTCAAGGATCTTACCGCTGTTGA
GATCCAGTTCGATGTAACCCACTCGTGCACCCAAGTATCTTCAGCATCTTTACTTTCA
CCAGCGTTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGG
CGACACGGAAATGTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAGCATTTATC
AGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAG
GGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAAATTTGTAAGCGTTAATATTTGTT
AAAAATTCGCGTTAAATTTTGTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGG
CAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTG
GAACAAGAGTCCACTATTAAGAAGCTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTA
TCAGGGCGATGGCCCACTACGTGATAACTTCGTATAATGTATGCTATACGAAGTTATCAC
TACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATC
GGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAGCCAACCTGGCTTATCG
AAATTAATACGACTCACTATAGGGAGACCGGC

pONY8.3G PGK + (SEQ ID No 48)

AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACGCGTTTGTGAGATTCTGTGCGC
GACTAAATTCATGTCGCGCGATAGTGGTGTATCGCCGATAGAGATGGCGATATTGGAA
AAATTGATATTTGAAAATATGGCATATTGAAAATGTGCGCGATGTGAGTTTCTGTGTAAC
TGATATCGCCATTTTCCAAAAGTGATTTTGGGCATACGCGATATCTGGCGATAGCGCT
TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGTC
GCAAAATATCGCAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCCGATAGAGG
CGACATCAAGCTGGCACATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
ATTAGCCATATTATTCATTGGTTATATAGCATAAAATCAATATTGGCTATTGGCCATTGCA
TACGTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACC
GCCCCACGACCCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAACTGCCCACTTGGCAGT
ACATCAAGTGATCATATGCCAAGTCCGCCCTATTGACGTCAATGACGGTAAATGGCC
CGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACACCAATGGGCGTGG
ATAGCGGTTGACTCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTT
GTTTGGCACCAAAATCAACGGGACTTTCAAAATGTGTAACAACCTGCGATCGCCCGCC
CCGTTGACGCAAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGCTCGT
TTAGTGAACCGGGCACTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGTGAAAAGG
CCTTTGTAATAATATAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTTCGAGATC
CTACAGTTGGCGCCGAACAGGGACCTGAGAGGGGGCGCAGACCCTACCTGTTGAACCTGG
CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAAGTTACAGAAGTCTTCTGGAGGTGTTT
CTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCTTTGACATTGGAGCAAGGCG
CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAATACTGGTAACTGT
AATTGGGCGCTAAGTCTAGTAGACTTATTTATGATACCAACTTTGTAAGAAAAGGAC
TGGCAGCTGAGGGATGTCAATTCCATTGCTGGAAGATGTAATCAGACGCTGTCAGGACAA
GAAAGAGAGGCCTTTGAAAGAACATGGTGGGCAATTTCTGCTGTAAAGATGGGCTCCAG
ATTAATAATGTAGTAGATGGAAGGCATCATTCCAGCTCCTAAGAGCGAAATATGAAAAG
AAGACTGCTAATAAAAAGCAGTCTGAGCCCTCTGAAGAATATCTCTAGAACTAGTGGATC
CCCCGGCTGCAGGAGTGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCAT
TAGCCATATTATTCATTGGTTATATAGCATAAAATCAATATTGGCTATTGGCCATTGCATA
CGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCAT
GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGC
CCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
GGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTAC
ATCAAGTGATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCAGTACATGACCTTATGGGACTTCTCTACTTGGCAGTACATCTACG
TATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACATCAATGGGCGTGGAT
AGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTTGT
TTTGGCACCAAAATCAACGGGACTTTCAAAATGTGTAACAACCTCGCCCACTTGAACGC
AAATGGGCGGTAGGCATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTATGTAACC
GTCAGATCGCCTGGAGACGCCATCCACGCTGTTTGAACCTCCATAGAAGACACCGGGACC
GATCCAGCTCCGCGGCCCAAGCTTGTGGGATCCACGGTCCGACCATGGTGAGCAA
GGGCGAGGAGCTGTTACCGGGGTGGTGGCCATCCTGGTCGAGCTGGACGGCGACGTAAA
CGGCCACAAGTTCAGCGTGTCCGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGAC

CCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGGCCACCCCTCGTGACCAC
CCTGACCTACGGCGTGCACTGCTTACGCCGTACCCCGACCACATGAAGCAGCAGCACTT
CTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTCAAGGACGA
CGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCAT
CGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTA
CAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGT
GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCA
GCAGAACACCCCATCGGGGACGGCCCGTGTCTGTGCCGACAACCACTACCTGAGCAC
CCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGTGGAGTT
CGTGACCGCCCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGCCCGCA
CTCTAGAGTCGACCTGCAGGCATGCAAGCTTCAGCTGCTCGAGGGGGGGCCCGGTACCCA
GCTTTTGTTCCTTTAGTGAGGGTTAATTGCGCGGGAAGTATTTATCACTAATCAAGCAC
AAGTAATACATGAGAACTTTTACTACAGCAAGCACAATCTCCAAAAAATTTGTTTTT
ACAAAAATCCCTGGTGAACATGATTGGAAGGGACCTACTAGGGTGTGTGGAAGGGTGATG
GTGCAGTAGTAGTTAATGATGAAGGAAAGGGAATAATTGCTGTACCATTAAACAGGACTA
AGTTACTAATAAAACCAAATTGAGTATTGTTGCAGGAAGCAAGACCCAACTACCATTGTC
AGCTGTGTTTCTGACCTCAATATTTGTTATAAGGTTTGATGAATCCAGGGGGAATC
TCAACCCCTATTACCCAACAGTCAGAAAAATCTAAGTGTGAGGAGAACACAATGTTTCAA
CCTTATTGTTATAAATGACAGTAAGAACAGCATGGCAGAATCGAAGGAAGCAAGAGAC
CAAGAATGAACCTGAAAGAAGAATCTAAAGAAGAAAAAGAAATGACTGGTGGAAAA
TAGGTATGTTTCTGTTATGCTTAGCAGGAACCTACTGGAGGAATACTTTGGTGGTATGAAG
GACTCCCACAGCAACATTATATAGGGTTGGTGGCGATAGGGGGAAGATTAAACGGATCTG
GCCAATCAAATGCTATAGAATGCTGGGGTTCCTTCCCGGGGTGTAGACCATTTCAAAATT
ACTTCAGTTATGAGACCAATAGAAGCATGCATATGGATAATAATACTGCTACATTATTAG
AAGCTTTAACCAATATAACTGCTCTATAAATAACAAAACAGAATTAGAAACATGGAAGTT
AGTAAAGACTTCTGGCATAACTCCTTTACCTATTCTTCTGAAGCTAACACTGGACTAAT
TAGACATAAGAGAGATTTTGGTATAAGTGCAATAGTGGCAGCTATTGTAGCCGCTACTGC
TATTGCTGCTAGCGCTACTATGTCTTATGTTGCTCTAACTGAGGTTAACAAAATAATGGA
AGTACAAAATCATACTTTTGGGTAGAAAATAGTACTCTAAATGGTATGGATTTAATAGA
ACGACAAAATAAGATATTATATGCTATGATTCTTCAAAACACATGCAGATGTTCAACTGTT
AAAGGAAAGACAACAGGTAGAGGAGACATTTAATTTAATTGGATGTATAGAAAGAACACA
TGTATTTTGTACTACTGGTCACTCCCTGGAATATGTCATGGGGACATTTAAATGAGTCAAC
ACAATGGGATGACTGGGTAAAGCAAAATGGAAGATTAAATCAAGAGATACTAACTACACT
TCATGGAGCCAGGAACAATTGGCACAATCCATGATAACATTCAATACACCAGATAGTAT
AGCTCAATTGGAAAAGACCTTTGGAGTCATATTGGAAATTGGATTCTGGATTGGGAGC
TTCCATTATAAAATATATAGTGATGTTTTGCTTATTTATTTGTTACTAACCTCTTCGCC
TAAGATCCTCAGGGCCCTCTGGAAGGTGACCAGTGGTGCAGGGTCTCCGGCAGTCGTTA
CCTGAAGAAAAAATCCATCACAAACATGCATCGCGAGAAGACACCTGGGACCAGGCCCA
ACACAACATACACCTAGCAGGCGTGACCGGTGGATCAGGGGACAAATACTACAAGCAGAA
GTACTCCAGGAACGACTGGAATGGAGAATCAGAGGAGTACAACAGGCGGCCAAAGAGCTG
GGTGAAGTCAATCGAGGCATTTGGAGAGAGCTATATTTCCGAGAAGACCAAAGGGGAGAT
TTCTCAGCCTGGGGCGGCTATCAACGAGCACAAGAACGGCTCTGGGGGGAACAATCCTCA
CCAAGGGTCTTAGACCTGGAGATTGGAAGCGAAGGAGGAAACATTTAGACTGTTGCAT
TAAAGCCCAAGAAGGAACCTCTCGCTATCCCTTGCTGTGGATTTCCCTTATGGCTATTTG
GGGACTAGTAATTATAGTAGGACGCATAGCAGGCTATGGATTACGTGGACTCGCTGTTAT
AATAAGGATTTGTATTAGAGGCTAAATTTGATATTTGAAATAATCAGAAAAATGCTTGA
TTATATTGGAAGAGCTTTAAATCCTGGCACATCTCATGTATCAATGCCTCAGTATGTTA
GAAAAACAAGGGGGGAACTGTGGGGTTTTATGAGGGGTTTTATAAATGATTATAAGAGT
AAAAAGAAAGTTGCTGATGCTCTATAACCTTGATAACCCAAAGGACTAGCTCATGTTG

CTAGGCAACTAAACCGCAATAACCGCATTGTGTACGCGAGTTCCTCCATTGGTGACGCGTT
AACTTCCTGTTTTACAGTATATAAGTGCTTGATTCTGACAATTGGGCACTCAGATTCT
GCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGGCCTTTGTAATAAATAAATTCTCTA
CTCAGTCCCTGTCTCTAGTTTGTCTGTTCGAGATCCTACAGAGCTCATGCCTTGGCGTAA
TCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATA
CGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCTAATGAGTGAGCTAACTCACATTA
ATTGCGTTGCGCTCACTGCCCGCTTCCAGTCGGGAAACCTGTCGTGCCAGAGTAGGCCG
CCTCGGCCAGATCTGGGGTTGGGGTTGCCCTTTTCCAAGGCAGCCCTGGGTTTGGCGAG
GGACGCGGCTGCTCTGGGCGTGGTTCGGGAAACGAGCGGCGCCGACCCTGGGTCTCGC
ACATTCTTCACGTCGCTTCGAGCGTCACCCGGATCTTCGCCGCTACCTTGTGGGCCCC
CCGGCAGCGTTCCTGCTCCGCCCTAAGTCGGGAAGGTTCTTGGGTTTCGGGCGTGC
CGGACGTGACAAACGGAAGCCGACGCTCTACTAGTACCCTCGCAGACGGACAGCGCCAG
GGAGCAATGGCAGCGCGCCGACCGCATGGGCTGTGGCCAATAGCGGCTGCTCAGCGGGG
CGCGCCGAGAGCAGCGGCGGGAAGGGCGGTGCGGGAGGCGGGGTGTGGGCGGTAGTG
TGGGCCCTGTTCTGCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTCG
GCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAGGCTAGCCTCGAGAAT
TCGCCACCATTGGCTGAGAGCAAGGAGGCCAGGGATCAAGAGATGAACCTCAAGGAAGAGA
GCAAAGAGGAGAAGCGCCGCAACGACTGGTGGAAGATCGACCCACAAGGCCCCCTGGAGG
GGGACCAGTGGTGCCGCGTGTGAGACAGTCCCTGCCGAGGAGAAGATTCTAGCCAGA
CCTGCATCGCCAGAAGACACCTCGGCCCGGTCCACCCAGCACACACCCTCCAGAAGGG
ATAGGTGGATTAGGGGCCAGATTTTGCAAGCCGAGGTCTCCAAGAAAGGCTGGAATGGA
GAATTAGGGGCGTGCAACAAGCCGCTAAAGAGCTGGGAGAGGTGAATCGCGCATCTGGA
GGGAGCTCTACTTCCGCGAGGACCAGAGGGGCGATTCTCCGCATGGGAGGCTACCAGA
GGGCACAAGAAAGGCTGTGGGGCGAGCAGAGCAGCCCCGCGTCTTGAGGCCCGGAGACT
CCAAAAGACGCCGCAACACCTGTGAAGTCGACCCGGCGCGGCTTCCCTTTAGTGAGG
GTTAATGCTTCGAGCAGACATGATAAGATACATTGATGAGTTTGACAAACCACAAGTAG
AATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAAC
CATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTATTTTATGTTTCAGGT
TCAGGGGGAGATGTGGGAGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAAAAT
CCGATAAGGATCGATCCGGGCTGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCC
CAACAGTTGCGCAGCCTGAATGGCGAATGGACGCGCCTGTAGCGGCGCATTAAAGCGCGG
CGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCGCTC
CTTTCGCTTTCTCCCTTCCCTTCTCGCCACGTTCCGCGGCTTTCCCGTCAAGCTCTAA
ATCGGGGGCTCCCTTTAGGGTTCCGATTTAGAGCTTTACGGCACCTCGACCGCAAAAAAC
TTGATTTGGGTGATGGTTACGTAGGCCGCTCGGCCGCGGGCATCACTGCATTAATG
AATCGGCCAACGCGCGGGAGAGGCGGTTTGGCTATTGGGCGCTCTTCCGCTTCTCGCT
CACTGACTCGCTGCGTCCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGC
GGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTATACTTCGT
ATAATGTATGCTATACGAAGTTATACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACC
GTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACA
AAAATCGACGCTCAAGTCAGAGGTGGCGAAAACCGACAGGACTATAAAGATACCAGGCGT
TTCCCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCCGACCCTGCCGCTTACCGGATACC
TGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATC
TCAGTTCCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCCGTTACG
CCGACCGCTGCGCCTTATCCGGTAACATCGTCTTGAGTCCAACCCGTAAGACACGACT
TATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTG
CTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTA
TCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCA
AACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAA

AAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACG
AAAACACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCC
TTTTAAATTAAAAATGAAGTTTAAATCAATCTAAAGTATATAGTAAACTTGGTCTG
ACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTCA
CCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTG
GCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTACCGGCTCCAGATTTATCAGCAA
TAAACGAGCCAGCCGAAGGGCCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCTCCA
TCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC
GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGGCTCGTCTTGGTATGGCTT
CATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAA
AAGCGGTTAGTCTCTTCGGTCTCCGATCGTTGTGAGAAAGTAAAGTTGGCCGAGTGTAT
CACTCATGGTTATGGCAGCACTGCATAATTCTTCTACTGTATGCCATCCGTAAGATGCT
TTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGA
GTTGCTCTTGCCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAG
TGCTCATCATTGGAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGA
GATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTACTTTCA
CCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGG
CGACACGGAATGTTGAATACTCATACTCTTCCTTTTCAATATTATTGAAGCATTATC
AGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAG
GGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAAATTGTAAGCGTTAATATTTTGT
AAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGG
CAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTG
GAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTA
TCAGGGCGATGGCCCACTACGTGATAACTTCGTATAATGTATGCTATACGAAGTTATCAC
TACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATC
GGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGAAAGCCAACCTGGCTTATCG
AAATTAATACGACTCACTATAGGGAGACCGGC

SEQ ID No 51

pONY3.2IREShyg

AGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTA
AGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGC CGGAGCAAAATT
5 TAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGG
CGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTTGACATTGATTATTGACT
AGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGC
GTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTG
10 ACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGACTTTCATTGACGTCAA
TGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCA
AGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTAC
ATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
ATGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTTGACTCACGGGGA
TTTCCAAGTCTCCACCCCAATTGACGTCAATGGGAGTTTGTGTTGGCACCAAAATCAACGG
15 GACTTTCACAAAATGTCGTAACAACCTGCCATCGCCCGCCCGTTGACGCAAAATGGGCGGTA
GGCGTGTACGGTGGGAGGCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCACTA
GAAGCTTTATTCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGTGCTTCTGACA
CAACAGTCTCGAACTTAAGCTGCAGTGACTCTCTTAAGGTAGCCTTGCAGAAGTTGGTCTG
TGAGGCACTGGGCAGGTAAGTATCAAGGTTACAAGCAGGTTTAAAGGAGACCAATAGAAA
20 CTGGGCTTGTGAGACAGAGAAGACTCTTGCCTTCTGATAGGCACCTATTGGTCTTACT
GACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCAGTTCAATTACAGCTCTTAA
GGCTAGAGTACTTAATACGACTCACTATAGGCTAGCCTCGAGGTCGACGGTATCGCCCGA
ACAGGGACCTGAGAGGGGGCGCAGACCCTACCTGTTGAACCTGGCTGATCGTAGGATCCCG
GGGACAGCAGAGGAGAACTTACAGAAGTCTTCTGGAGGTGTTCTGGCCAGAACACAGGA
25 GGACAGGTAAGATGGGAGACCCCTTTGACATGGAGCAAGGCGCTCAAGAAGTTAGAGAAAG
TGACGGTACAAGGGTCTCAGAAATTAACCTACTGGTAAGTGAATGGGCGCTAAGTCTAG
TAGACTTATTTTCATGATACCAACTTTGTAAAAGAAAAGGACTGGCAGCTGAGGGATGTCA
TTCCATTGCTGGAAGATGTAACCTAGACGCTGTGAGACAAGAAAGAGAGGCCCTTTGAAA
GAACATGGTGGGCAATTTCTGCTGTAAGATGGGCTCCAGATTAATAATGTAGTAGATG
30 TAAATGAAGCTAGTCAAACTTATTGGGATATTATCAGTAGACTGTACTTCTGAAGAAA
AGTCTGAGCCCTCTGAAGAAATATCCAATCATGATAGATGGGCTGGAAACAGAAATTTTA
GACCTTAACACCTAGAGGATATACTACTTGGGTGAATACCATACAGACAAATGGTCTAT
TAAATGAAGCTAGTCAAACTTATTGGGATATTATCAGTAGACTGTACTTCTGAAGAAA
TGAATGCATTTTGGATGTGGTACCTGGCCAGGCAGGACAAAAGCAGATATTACTTGATG
35 CAATTGATAAGATAGCAGATGATTGGGATAATAGACATCCATTACCGAATGTCTCCACTGG
TGGCACCCACCAAGGGCCCTATCCCATGACAGCAAGGTTTATTAGAGGTTTAGGAGTAC
CTAGAGAAAGACAGATGGAGCCTGCTTTTGATCAGTTTAGGCAGACATATAGACAAATGGA
TAATAGAAGCCATGTCAAGGCATCAAAAGTATGATTGGAACCTAAAGCTCAAAATA
TAGGCAAGGAGCTAAGGAACCTTACCCAGAAATTTGTAGACAGACTATTATCCCAATAA
40 AAAGTGAGGGACATCCACAAGAGATTTCAAAATCTTGAAGTATACACTGACTATTTCAGA
ACGCAATGAGGAAATGTAGAAATGCTATGAGACATTTAAGACCAGAGGATACATTAGAAG
AGAAAATGTATGCTTGCAGAGACATTGGAACTACAAAACAAAAGATGATGTTATTGGCAA
AAGCACTTCAGACTGGTCTTGGGGCCCATTTAAAGGTGGAGCCTTGAAGGAGGGCCAC
TAAAGGCAGCACAAACATGTTATACTGTGGGAAGCCAGGACATTTATCTAGTCAATGTA
45 GAGCACCTAAAGTCTGTTTTAAATGTAAACAGCCTGGACATTTCTCAAAGCAATGCAGAA
GTGTTCCAAAAACGGGAAGCAAGGGGCTCAAGGGAGGCCCCAGAAACAACTTTCCCGA
TACAACAGAAGAGTCAGCACAAACAAATCTGTTGTACAGAGACTCCTCAGACTCAAAATC
TGTACCCAGATCTGAGCGAAATAAAAAAGGAATACAATGTCAAGGAGAAGGATCAAGTAG
AGGATCTCAACCTGGACAGTTTGTGGGAGTAACATATAATCTAGAGAAAAGGCCTACTAC
50 AATAGTATTAATTAATGATCTCCCTTAAATGTACTGTTAGACACAGGAGCAGATCTTC
AGTGTGACTACTGCACATTATAATAGGTTAAAAATAGAGGGAGAAAAATATCAAGGGAC
GGGAATAATAGGAGTGGGAGGAAATGTGGAAACATTTTCTACGCTGTGACTATAAAGAA
AAAGGTAGACACATTAGACAAAGATGCTAGTGGCAGATATTCCAGTGACTATTTGGG
ACGAGATATTCTCAGGACTTAGGTGCAAAATTTGGTTTGGCACAGCTCTCCAGGAAAT
55 AAAATTTAGAAAAATAGAGTTAAAAAGGGCCACAAATGGGGCCAAAAATTCCTCAATGGCC
ACTCACTAAGGAGAACTAGAAGGGGCCAAAGAGATAGTCCAAAGACTATTGTGAGAGGG
AAAAATATCAGAAGCTAGTGACAATAATCCTTATAATTCACCCATATTTGTAATAAAAAA
GAGGCTGGCAAAATGAGGTTATTACAGATCTGAGAGAATTAACAAAAACAGTACAAGT
AGGAACGGAAATATCCAGAGGATTGCCTCACCCGGGAGGATTAATTAATGTAACACAT
60 GACTGTATTAGATATTGGAGATGCATATTTCACTATACCCTTAGATCCAGAGTTTAGACC
ATATACAGCTTCTACTATTCCCTCCATTAATCATCAAGAACAGATAAAAGATATGTGTG
GAAATGTTTACCACAAGGATTGCTGTTGAGCCCATATATATCAGAAAACATTACAGGA
AATTTTACAACCTTTTAGGGAAAGATATCCTGAAGTACAATTGTATCAATATATGGATGA

TTTGTTCATGGGAAGTAATGGTTCTAAAAAACACACAAAGAGTTAATCATAGAATTAAG
GGCGATCTTACTGGAAAAAGGTTTTGAGACACCAGATGATAAATTACAAGAAGTGCCACC
TTATAGCTGGCTAGGTTATCAACTTTGTCTGAAAAATTGGAAAGTACAAAAATGCAATT
AGACATGGTAAAGAAATCCAAACCTTAATGATGTGCAAAAAATTAATGGGGAATATAACATG
5 GATGAGCTCAGGGATCCCAGGCTTGACAGTAAACACATTGCAAGCTACTACTAAGGGATG
TTTAGAGTTGAATCAAAAAGTAATTTGGACGGAAGAGGCACAAAAAGAGTTAGAAGAAAA
TAATGAGAAGATTAAAAATGCTCAAGGGTTACAATATTATAATCCAGAGAAGAAATGTT
ATGTGAGGTTGAATTAACAAAAATTAATGAGGCAACTTATGTTATAAAACAATCACAAGG
AATCCTATGGGCAGGTAAAAAGATTATGAAGGCTAATAAGGGATGGTCAACAGTAAAAAA
10 TTTAATGTTATTGTTGCAACATGTGGCAACAGAAAGTATTACTAGAGTAGGAAAAATGTCC
AACGTTTAAGGTACCATTTACCAAGAGCAAGTAATGTGGGAAATGCAAAAAGGATGGTA
TTATCTTGGCTCCCAAGAAATAGTATATACACATCAAGTAGTTCATGATGATTGGAGAT
GAAATTTGGTAGAAGAACCTACATCAGGAATAACAATATACACTGATGGGGGAAAAACAAA
TGGAGAAGGAATAGCAGCTTATGTGACCAGTAATGGGAGAACTAAACAGAAAAGGTTAGG
15 ACCTGTCACTCATCAAGTTGCTGAAAGAAATGGCAATACAAATGGCATTAGAGGATACCAG
AGATAACAAGTAAATATAGTAACGTGATGTTATTATTGTTGAAAAATATTACAGAAGG
ATTAGGTTTAGAAGGACCACAAAGTCTTGGTGGCTATAATACAAAAATACAGAGAAAA
AGAGATAGTTTATTGTTGGTACCTGGTACAAAGGGATATATGTTAATCAATTGGC
AGATGAAGCCGCAAAAAATAAAGAAAGAAATCATGCTAGCATACCAAGGCACACAAATTA
20 AGAGAAAAGAGATGAAGATGCAGGTTTTGACTTATGTGTTCTTATGACATCATGATACC
TGATCTGACACAAAAATCATACCCACAGATGTAAAAAATCAAGTTCTCTAATAGCTT
TGGATGGGTCAGTGGGAAATCATCAATGGCAAAACAGGGTTATTAATTAATGGAGGAAT
AATTGATGAAGGATATACAGGAGAAAAACAAGTGATATGTAATAATTGGAAAAAGTAA
TATTAAATTAATAGAGGGACAAAAATTTGCAAAATTAATTACTACGCATCACTCAA
25 TTCCAGACAGCCTTGGGATGAAAAATAAATATCTCAGAGAGGGGATAAAGGATTTGGAAG
TACAGGAGTATTCTGGGTAGAAAAATTTACAGGAAGCACAAGATGAACATGAGAAATGGCA
TACATCACCAGAAATATTGGCAAGAAATTAAAGATACCATTTGACTGTAGCAAAACAGAT
AACTCAAGAAATGTCTCTATTGCACTAAGCAAGGATCAGGACCTGCAGGTTGTGTATGAG
ATCTCCTAATCATTGGCAGGCAGATTGCACACATTTGGACAATAAGATAATATTGACTTT
30 TGTAGAGTCAAAATCAGGATACATACATGCTACATTATTGTCAAAAGAAAAATGCATTATG
TACTTCATTGGCTATTTTAGAATGGGCAAGATTGTTTTACCAAAGTCTTACACACAGA
TAACGGCACTAATTTGTGGCAGAACCAAGTTGTAATTTGTTGAAGTTCTAAAGATAGC
ACATACCACAGGAATACCATATCATCCAGAAAGTCAGGGTATTGTAGAAAGGGCAAAATAG
GACCTTGAAGAGAGAAGATTCAAGTCATAGAGACAACACTCAAACTGGAGGCAGCTTT
35 ACAACTTGCTCTCTACTTGTAAACAAAGGGAGGGAAAGTATGGGAGGACAGACACCATG
GGAAGTATTTACTACTAATCAAGCACAAGTAATACATGAGAACTTTTACTACAGCAAGC
ACAATCTCCCAAAAAATTTGTTTTTACAAAATCCCTGGTGAACATGATTGGAAGGGACC
TACTAGGGTGTGTGGAAGGGTGATGGTGCAGTAGTAAATGATGAAGGAAAGGGAAAT
AATTGCTGTACCATTAACCAAGGACTAAGTTACTAATAAAACCAAAATGAGTATTGTTGCA
40 GGAAGCAAGACCAACTACCATTTGTCAGCTGTGTTTCTGACCTCAATATTGTTATAAG
GTTTGATATGAATCCAGGGGGAATCTCAACCCCTATTACCAACAGTCAGAAAAATCTA
AGTGTGAGGAGAACACAATGTTTCAACCTTATTGTTATAATAATGACAGTAAGAACAGCA
TGGCAAGAAATCGAAGGAAGCAAGAGACCAAGAAATGAACCTGAAAGAAAGAACTAAAGAA
45 AAAAAAGAAAGAAATGACTGGTGGAAATAGGTATGTTTCTGTTATGCTTAGCAGGAACTA
CTGGAGGAATACTTTGGTGGTATGAAGGACTCCACAGCAACATTATATAGGTTGGTGG
CGATAGGGGGAAGATTAAACGGATCTGGCAATCAAAATGCTATAGAATGTGGGTTCTT
TCCCGGGGTGTAGACATTTCAAAATTACTTCAGTTATGAGACCAATAGAAGCATGCATA
TGGATAATAATAGTGTACATTATTAGAAGCTTTAACCATATAACTGCTCTATAAATAA
50 CAAACAGAAATTAGAAACATGGAAGTTAGTAAAGACTCTGGCATAACTCCTTTACCTAT
TTCCTCTGAAGCTAACACTGGACTAATTAGACATAAGAGAGATTTGGTATAAGTGCAAT
AGTGGCAGCTATTGTAGCCGCTACTGCTATTGCTGCTAGCGCTACTATGCTTATGTTGC
TCTAACTGAGGTTAAACAAAATATGGAAGTACAAAATCATACTTTTGAGGTAGAAAAATAG
TACTCTAAATGGTATGGATTTAATAGAACGACAAATAAGATATTATATGCTATGATTCT
TCAAACACATGCAGATGTTCAACTGTTAAAGGAAAGCAACAGGTAGAGGAGACATTAA
55 TTAATTGGATGTATAGAAAGAACACATGTATTTGTCTACTGGTCACTCCCTGGAATAT
GTCATGGGGACATTTAAATGAGTCAACACAATGGGATGACTGGGTAAGCAAAATGGAAGA
TTTAAATCAAGAGATACTAACTACACTTCATGGAGCCAGGAACAATTTGGCACAATCCAT
GATAACATTCAATACACCAGATAGTATAGCTCAATTTGAAAAAGACCTTTGGAGTCATAT
TGGAAATTTGATTCTGGATTGGGAGCTTCCATTAAAAATATATAGTATGTTTGTCT
60 TATTTATTTGTTACTAACCTCTTCGCCTAAGATCCTCAGGGCCCTCTGGAAGGTGACCAG
TGGTGCAAGGTCCTCCGGCAGTCTGTTACCTGAAGAAAAATTCATCACAACATGCATC
GCGAGAAGACACTGGGACCAGGCCCAACACAACATACACTAGCAGGCGTGACCGGTGG
ATCAGGGGACAAATACTACAAGCAGAACTACTCAGGAACGACTGGAATGGAGAATCAGA
GGAGTACAACAGGCGCCAAAGAGCTGGGTGAAGTCAATCGAGGCATTTGGAGAGAGCTA
65 TATTTCCGAGAAGACCAAGGGGAGATTTCAGCTGGGGCGGCTATCAACGAGCACAA
GAACGGCTCTGGGGGAACAATCCTCACCAGGGTCTTAGACTGGAGATTGGAAGCGGA
AGGAGGAAACATTTAGCTGTTGCATTAAAGCCCAAGAGGAAGTCTCGCTATCCCTTG
CTGTGGATTTCCCTTATGGCTATTTTGGGGTGCACCCGGGGCGGCTAGAGGAATT
CGCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAGCCGCTTGGAAATAGGCGCG
70 TGTGTGTTGTCTATATGTGATTTCCACCATATTGCCGTCTTTGGCAATGTGAGGGC
CGGAAACCTGGCCCTGCTCTTGTGACGAGCATTCCTAGGGGTCTTCCCTCTCGCCAAA
GGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTCTCTGGAAGCTCTTGAAGA
CAAAACAGCTGTAGCGACCTTTGCAAGGACGGGAACCCCACTGGCGACAGGTGC
CTCTGGCGCCAAAAGCCACGTGTATAAGATACACTGCAAAAGGCGGCACAACCCAGTGC

CACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTAGTCAAC
AAGGGGCTGAAGGATGCCAGAAAGGTACCCATTGTATGGGAATCTGATCTGGGGCCTCG
GTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAGCTCTAGGCCCCCCGAACAC
GGGGACGTGGTTTTCTTTGAAAAACACGATGATAAGCTTGCCACAACCCCGTACCAAAG
5 ATGGATAGATCCGGAAGCCTGAACACCGCGACGCTGTGCGAGAAGTTTCTGATCGAA
AAGTTCGACAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCOTGCTTTC
AGCTTCGATGTAGGAGGGCGTGGATATGCTCTGCGGGTAAATAGCTGCGCCGATGGTTTC
TACAAAGATCGTTATGTTATCGGCACCTTTGCATCGGCCGCGCTCCCGATTCCGGAAGTG
10 CTTGACATTGGGGAATTACGCGAGAGCCTGACCTATTGCATCTCCCGCCGTGCACAGGGT
GTCACGTTGCAAGACCTGCCTGAAACCGAACTGCCCGCTGTTCTGCAGCCGGTCCGCGAG
GCCATGGATGCGATCGCTGCGGCCGATCTTAGCCAGACGAGCGGGTTCGGGCCATTCCGA
CCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCGATTGCTGATCCC
CATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAGTGCCTCGTCCGCGAGGCT
CTCGATAGCTGATGCTTTGGGCGGAGGACTGCCCGAAGTCCGGCACCTCOTGCACGCG
15 GATTTCCGCTCCAACAATGCTCTGACGGACAATGGCCGATAACAGCGGTCAATTGACTGG
AGCGAGGGCATGTTCCGGGATTCCCAATACGAGGTCCGCAACATCTTCTTCTGAGGCCG
TGGTTGGCTTGTATGGAGCAGCAGACCGCTACTTCGAGCGGAGGCGATCCGGAGCTTGCA
GGATCGCCCGCGCTCCGGCGTATATGCTCCGATTTGGTCTTGACCAACTCTATCAGAGC
20 TGGTTGACGGCAATTTGATGATGCGAGTTGGGCGCAGGGTCGATGCGACGCAATCGTC
CGATCCGAGCGGGGACTGTCGGCGGTACACAAATCGCCCGCAGAAGCGCGGCCGTCTCG
ACCGATGGCTGTGTAGAGTACTCGCCGATAGTGGAAACCGACGCCACGACTCGTCCG
AGGGCAAAAGGAATAGAGTAGATGCCGACCGAACAAGAGCTGATTTGAGAGAAGCCTCAGC
CAGCAACTCGCGGAGCCTAGCAAGGCAATGCGAGAGAACGGCCCTACGCTTGGTGGCA
CAGTTCTCGTCCACAGTTCGTAAGCTCGCTCGGCTGGGTCCGGGAGGGCCGGTCCGAG
25 TGATTCAGGCCCTTCTGGATTGTGTTGGTTCGCCAGGGCAGGATTGTCATGCCACGCACT
CGGGTGTCTGACTGATCCCGCAGATTGGAGATCGCCGCCGTGCCTGCCGATTGGGTGC
AGATCTAGAGCTCGCTGATCAGCCTCGACTGTGCTCTAGTTGCCAGCCATCTGTTGTT
GCCCTCCCGCTGCTTCTGAGCGGAAAGAACCAAGCTGGGGCTCGAGTGCAATCTAGTTG
AAAATGAGGAAATTCATCGCATTTGTCTGAGTAGGTGTCTATTCTTGGGGGTGGGG
30 TGGGCGTAGCAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCCG
TGGCTCTATGGCTTCTGAGCGGAAAGAACCAAGCTGGGGCTCGAGTGCAATCTAGTTG
GGTTGTCCAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTTAGCTAG
AGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATGTTATCCGCTCACAATT
35 CCACACAACATAGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGC
TAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCAGTCCGGAAACCTGTCGTGC
CAGGTGCAATTAATGAATCGGCCAACCGCGGGGAGAGGGCGTTTTCGCTATTGGGCGCTCT
TCCGCTTCCCTCGCTCACTGACTCGCTCGCTCGCTCGGTCGTTCCGGTCCGGCGAGCGGTATCA
GCTCACTCAAAGGGCGGTAAACGGTTATCCACAGAATCAGGGGATAACCGAGGAAAGAAC
40 ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTT
TTCCATAGGCTCCGCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGG
CGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCGCTGGAAGCTCCCTCGTGCGC
TCTCTGTTCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGC
GTGGCGCTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCCGGTGTAGGTGCTTCCGTCC
45 AAGCTGGGCTGTGTGCACGAACCCCGCTTACGCCGACCGCTGCGCCTATCCGGTAAC
TATCGTCTTGAGTCAACCCCGTAAGACACGACTATCGCCACTGGCAGCAGCCACTGGT
AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
AACTACGGCTACACTAGAAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACC
TTCGGAAGAAAGAGTTGGTAGCTCTTGATCCGCGCAACAAACACCGCTGGTAGCGGTGGT
50 TTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAAGATCCTTTG
ATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACTACGTTAAGGGATTTTGGTC
ATGAGATTATCAAAAGGATCTTACCTAGATCCTTTAAATTAATAATGAAGTTTAAAT
TCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
GCACCTATCTCAGCGATCTGTCTATTTCGTTTATCCATAGTTGCCTGACTCCCGCTCGTG
55 TAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGA
GACCCACGCTACCGGCTCCAGATTATCAGCAATAAACACGCGAGCCGGAAGGGCCGAG
CGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAAATTGTTGCCGGAA
GCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGGC
ATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCAATCAGCTCCGGTTCCTCAACGATCA
60 AGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGTTAGTCTCTTCGTTCTCCG
ATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCAT
AATTCCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAAGTCAACC
AAGTCAATCTGAGAAATAGTGTATGCGGCGACCGAGTTGCTCTTCCCGCGCTCAATACGG
GATAATACCGCGCCACATAGCAGAATTTAAAGTGTCTATCATTGGAACGTTCTTCG
GGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCCGATGAACCACTCGT
65 GCACCAACTGTCTTACGATCTTTTACTTTCACAGCGTTTCTGGGTGAGCAAAAAACA
GGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACATATA
CTCTTCTTTTCAATATTATGAAGCAATTTACAGGGTTATTGTCTCATGAGCGGATAC
ATATTGAATGTATTGAAGAAATAAACAATAGGGGTTCGCGCACATTTCCCGGAAAA
GTGCCACCTGACGTGACGAGATCGGG

70

pONY8ZA CMVHyb (SEQ ID No 52)

AGATCTTGAATAATAAAATGTGTGTTTGTCCGAAATACCGGTTTTGAGATTCTGTCCG

GACTAAATTCATGTCGCGGATAGTGGTGTTCATCGCCGATAGAGATGGCGATATTGGAA
AAATTGATATTGAAAAATATGGCATATTGAAAAATGTCGCGGATGTGAGTTTCTGTGTAAC
TGATATCCCATTTTTTCCAAAAGTGATTTTTGGGCATACGCGATATCTGGCGATAGCGCT
TATATCGTTTACGGGGGATGGCGATAGACGACTTTGGTGACTTGGCGGATTTCTGTGTGTC
5 GCAAAATATCGCAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCGATAGAGG
CGACATCAAGCTGGCACATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
ATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCA
TAGCTTGTATCCATATCGTAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC
ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAATTAGTTCA
10 TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACC
GCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTAAATGGCC
CGCTTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCTACTTGGCAGTACATCTA
15 CGTATTGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACACCAATGGCGGTGG
ATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCTTACGCTCAATGGGAGTTT
GTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTGCGATCGCCCGCC
CCGTTGACGCAAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGCTCGT
TTAGTGAACCGGGCACTCAGATTCTGCGGTCTGAGTCCCTCTCTGCTGGGCTGAAAAGG
20 CCTTTGTAAATAATATAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTGAGATC
CTACAGTTGGCGCCCGAACAGGGACCTGAGAGGGGCGCAGACCCTACCTGTTGAACCTGG
CTGATCGTAGGATCCCCGGGACAGCAGAGGAGAAGTTACAGAAGTCTTCTGGAGGTGTTT
CTGGCCAGAACACAGGAGGACAGGTAAGATTGGGAGACCCTTTGACATTGGAGCAAGGCC
CTCAAGAAGTTAGAGAAGGTGACGGTACAAGGGTCTCAGAAATTAACACTGTTAACTGT
25 AATTGGGCGCTAAGTCTAGTAGACTTATTTCAATGATACCAACTTTGTAAGAAAAGGA
CTGGCAGCTGAGGGATTGTCATTCCATTGCTGGAAGATTGTAACCTCAGACGCTGTCAAGGA
CAAGAAAGAGAGGCCCTTTGAAAGAACATTGGTGGGCAATTTCTGCTGAAGATTGGGCC
TCCAGATTAAATAATTGTAGTAGATTGGAAGGCATCTTCCAGCTCCTAAGAGCGAAATA
TTGAAAGAAAGACTGCTAATAAAAGCAGTCTGAGCCCTGGAAGAAATATCTCTAGAAGT
30 AGTGGATCCCCGGGCTGCAAGAGTGGGGAGGACGATGGCCGCTTGGTGGAGGCGGAT
CCGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCC
ATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATT
ACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAAT
AGTTCAATGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGG
35 CTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAAC
GCCAATAGGAGCTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCCACTT
GGCAGTACAGTCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA
ATGGCCCGCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTA
CATCTGAGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACATCAATGG
40 GCGTGAGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCTTACGCTCAATGG
GAGTTTGTGTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCC
ATTGACGCAAAATGGGCGGTAGGCGATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTT
AGTTCAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACA
45 CCGGGACCGATCCAGCCTCCGCGGCCCAAGCTTCAGCTGCTCGAGGATCTGCGGATCCG
GGGAATTCCCGAGTCTCAGGATCCACCATGGGGATCCCGTCTTTTACAACGTCGTGAC
TGGGAAAACCTGGCGTTACCCAACCTAATCGCCTTGACGACATCCCGCTTTCCGACG
TGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAAT
GGCGAATGGCGCTTTGCGTGTGTTCCGGCACAGAAAGCGGTGCGGAAAGCTGGCTGGAG
TGGCATCTTCTGAGGCCGATACTGTCGTCGTCCTCCCAACTGGCAGATGCACGGTTAC
50 GATGCGCCCATCTACACCAACGTAACCTATCCCATTTACGGTCAATCCGCGGTTTGTCCC
ACGGAGAAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAGCTGGCTACAG
GAAGGCCAGACGCAATTATTTTGTGCGGTTAACTCGCGGTTTCACTGTGGTGCAAC
GGCGCTGGGTGCGTTACGGCCAGGACAGTCTGTTGCCGCTGGAATTGACCTGAGCGCA
TTTTTACGCGCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCGTTGGAAGTACGGCAGT
55 TATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTCCGTGACGTCTCGTTGCTG
CATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTAATGATGATTTT
AGCCGCGCTGTACTGGAGGCTGAAGTTTCAATGTCGCGGAGTTGCGTGACTACCTACGG
GTAACAGTTTCTTATGGCAGGGTGAACCGCAGGTGCGCACGCGCACCGCGCTTTCCGG
GGTGAATTTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGCTGAACGTC
60 GAAAACCGGAACTGTGGAGCGCGGAAATCCCGAATCTCTATCGTGGCGGTGGTTGAAGT
CACACCGCGACGGCAGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTG
CGGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTGAGGGCTTAAC
CGTCACGAGCATCATCTCTGATGTCAGGTGATGGATGAGCAGACGATGGTGACAGGAT
ATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCCGATTATCCGAACCAT
65 CCGCTGGGTACACGCTGTGCGACCGCTACGGCCTGTATGTTGGTGAAGGCAATATT
GAAACCCACGGCATGGTGCCAAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCG
ATGAGCGAACCGGTAACCGCAATGGTGACGGCGATCGTAATCACCCGAGTGTGATCATC
TGGTCCGTGGGGAATGAATCAGGCCACGGCGCTAATCAGCAGCGCTGTATCGCTGATC
AAATCTGTCGATCTTCCCGCCCGGTGCAATGAAGGGCGGAGCCGACACCGCGCC
70 ACCGATATTATTGCCCGATGTACGCGCGGTGGATGAAGACAGCCCTTCCCGCTGTG
CCGAAATGGTCTTACATAAAAAATGGCTTTCCGCTACCTGGAGAGACGCGCCGCTGATCCT
TGCGAATACGCCACCGCATGGTAACAGTCTTGGCGGTTTCCGCTAAATCTGGCAGGCG
TTTCTGTCAGTATCCCGGTTACAGGGCGGCTTCTGCTGGGACTGGGTGGATCAGTCCGCT
ATTAATATGATGAAAACGGCAACCCGTGGTCCGCTACGGCGGTGATTTTGGCGATACG

CCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCA
GCGCTGACGGGAAGCAAAACACCAGCAGCAGTTTCCAGTTCGTTTATCCGGGCAAAACC
ATCGAAGTGACCCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCTGCACTGGATG
GTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGCTCCACAA
5 GGTAAACAGTTGATTGAACCTGCCTGAACACCGCAGCCGGAGAGCCGGGCAACTCTGG
CTCACAGTACGCGTAGTGCAACCGAACCGCAGCGCATGGTCAGAAGCCGGGCACATCAGC
GCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCGCTCCAC
GCCATCCCCATCTGACCACCAGCGAAATGGATTTTGCATCGAGCTGGTAATAAGCGT
TGGCAATTTAACCGCCAGTCAGGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAA
10 CTGCTGACGCCGCTGCGGATCAGTTACCCGTCACCGCTGGATAACGACATTGGCGTA
AGTGAAGCGACCCGATTGACCTAACCGCTGGTTCGAACGCTGGAAGCCGGCGGCCAT
TACCAGCCGGAAGCAGCGTGTGTCAGTGCACGGCAGATACACTTGTGATGCGGTGCTG
ATTACGACCGCTCAGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACC
TACCGGATTGATGGTAGTGGTCAAAATGGCGATTACCGTTGATGTGAAGTGGCGAGCGAT
15 ACACCGCATCGCGCGGCGGATTGGCCTGAACCTGCCAGCTGGCGCAGGTAGCAGAGCGGTA
AACTGGCTCGGATTAGGGCCGCAAGAAAATATCCCGACCGCTTACTGCCGCTGTTTT
GACCGTGGGATCTGCCATTGTGACAGATGTATACCCGTCAGCTTCCCGAGCGAAAAC
GGTCTGCGCTGCGGGACGCGCAATTGAATTATGGGCCACACCAAGTGGCGCGGCGACTTC
CAGTTCAACATCAGCCGCTACAGTCAACAGCAACTGATGGAACCAGCCATCGCCATCTG
20 CTGACCGCGGAAGAGGCACATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGC
GACGACTCTGGAGCGTACGTATCGCGGAAATCCAGCTGAGCGCCGCTGCTACCAT
TACCAGTTGGTCTGGTGTCAAAAATAATAAACCGGGCAGGGGGATCCGCAGATCCGG
CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGAGGCAAGAGT
ATGCAAGCATGCTCGAGGAATTCGATATCAAGCTTATCGATACCGTGCACCTCGAGGG
25 GGGGCCCGGTACCCAGCTTTTGTCTCCTTAGTGAGGGTTAATTGCCGGGAAGTATTTA
TCACTAATCAAGCACAAAGTAATACATGAGAACTTTTACTACAGCAAGCACAATCTCCA
AAAAATTTGTTTTACAAAATCCCTGGTGAACATGATTGGAAGGGACCTACTAGGGTGC
TGTGGAAGGGTATGGTGCAGTAGTATTAATGATGAAGGAAAGGAATAATTGCTGTAC
CATTAACCAAGGACTAAGTTACTAATAAAACCAAAATTGAGTATTGTTGCAGGAAGCAAGAC
30 CCAACTACCATTTGTGAGCTGTGTTTCTGACCTCAATATTTGTTATAAGGTTTGATATGA
ATCCCAAGGGGAATCTCAACCCCTATTACCAACAGTCAGAAAAATCTAAGTGTGAGGAG
AACACAATGTTTCAACCTTATTGTTATAAATGACAGTAAGAACAGCATGGCAGAATCG
AAGGAAGCAAGAGACCAAGAATGAACCTGAAAGAAGAACTAAAGAAGAAAAAGAAGAA
35 ATGACTGGTGGAAAAATAGGTATGTTTCTGTTATGCTTAGCAGGAACACTGGAGGAATAC
TTTGGTGGTATGAAGGACTCCACAGCAACATTATATAGGGTTGGTGGCGATAGGGGGAA
GATTAACCGGATCTGGCAATCAAAATGCTATAGAATGCTGGGGTCTCTCCCGGGGTGA
GACCAATTGAAAAATCTTCAAGTTATGAGACCAATAGAAGCATGCATATGGATAATAATA
CTGCTACATTATTAGAAGCTTAAACCAATATAACTGCTCTATAAATAACAAAACAGAATT
40 AGAAAATGGAAGTTAGTAAAGACTTCTGCATAACTCCTTACCTATTTCTTCTGAAGC
TAACACTGGAGTAATTAGACATAAAGAGAGATTTGGTATAAGTGAATAGTGGCAGCTAT
TGTAGCCGCTACTGCTATTGCTGCTAGCGCTACTATGCTTATGTTGCTCTAAGTGGT
TAACAAAATAATGGAAGTACAAAATCATACTTTGAGGTAGAAAATAGTACTCTAAATGG
TATGGATTATAAGAACGACAAAATAAAGATATTATATGCTATGATTCTTCAACACATGC
45 AGATGTTCAACTGTTAAAGGAAAGACAACAGGTAGAGGAGACATTAATTTAATTGGATG
TATAGAAAGAACACATGTATTTGTCTACTGGTCACTCCCTGGAATATGTCATGGGGACA
TTTAAATGAGTCAACACAATGGGATGACTGGGTAAGCAAAATGGAAGATTAAATCAAGA
GATACTAACTACACTTCATGGAGCCAGGAACAATTTGCCACAATCCATGATAACATTCAA
TACACCGATAGTATAGCTCAATTTGGAAAAAGACCTTTGGAGTCAATTGGAAATTGGAT
50 CCTGGATTGGGAGCTTCCATTATAAAATATATAGTGATGTTTTTGCTTATTTATTTGTT
ACTAACCTCTTCGCTAAGATCCTCAGGGCCCTCTGGAAGGTGACCAAGTGTGCAAGGGT
CTCCGGGAGTCTTACCTGAAGAAAAATTCATCACAAACATGCATCGCGAGAAGACAC
TGGGACCAAGGCCAACACAACATACACCTAGCAGGCGTGACCGGTGGATCAGGGGACAA
55 ATACTACAAGCAGAAGTACTCCAGGAACGACTGGAATGGAGAATCAGAGGAGTACAACAG
GCGGCCAAAGAGCTGGGTGAAGTCAATCGAGGCATTGGAGAGAGCTATATTTCCGAGAA
GACCAAAAGGGGAGATTCTCAGCCTGGGGCGGTATCAACGAGCACAAGAACCGCTCTGG
GGGGAACAATCCTCACCAAGGGTCTTAGACCTGGAGATTGGAAGCGAAGGAGGAAACAT
TTATGACTGTTGCATTAAGCCCAAGGAAGTCTCGCTATCCCTTGTGTGGATTTC
CTTATGGCTATTTTGGGACTAGTAATTATAGTAGGACGATAGCAGGCTATGGATTACG
60 TGGACTCGCTGTTATAATAAGGATTGTATTAGAGGCTTAAATTTGATTTGAAATAAT
CAGAAAAATGCTTGATTATTTGGAAGAGCTTTAAATCCTGGCAGATCTCATGTATCAAT
GCCTCAGTATGTTAGAAAAACAAGGGGGAACCTGGGGTTTTATGAGGGGTTTTATA
AACTGCAGGAGTGGGAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCATTAGCC
ATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTG
TATCCATATCATAATATGTACATTTATTTGGCTCATGTCCAACATTACCGCATGTTGA
65 CATTGATTATGACTAGTTATTAATAGTAATCAATTACGGGGTCAATAGTTTATAGCCCA
TATATGGAAGTCCGCGTTACATAACTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC
GACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACT
TTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGG
70 CATTATGCCCACTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTA
GTACATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGG
TTTGACTACGGGGATTTCAAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTTGG
CACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCATTTGACGCAATG
GGCGGTAGGCATGTACGGTGGGAGGTCTATATAACGAGAGCTCGTTTATGTAACCGGCA

CTCAGATTCTGCGGTCTGAGTCCCTTCTCTGCTGGGCTGAAAAGGCCTTTGTAATAATA
TAATTCTCTACTCAGTCCCTGTCTCTAGTTTGTCTGTTCGAGATCCTACAGAGCTCATGC
CTTGGCGTAATCATGGTCATAGCTGTTCCCTGTGTGAAATTGTTATCCGCTCACAATTCC
5 ACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCAGTCGGGAAACCTGTCTGTCCA
GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGCGCTCTTC
CGCTTCTCGCTCACTGACTCGCTCGCTCGGTCTCGGCTCGGCGAGCGGTATCAGC
TCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACAT
10 GTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTT
CCATAGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGACGCTCAAGTCAGAGGTGGCG
AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGGCGTC
TCCTGTTCCGACCTGCGCTTACCGGATACCTGTCCGCTTTCCTCCCTCGGGAAGCGT
GGCGCTTTCATAGCTACGCTGTAGGTATCTCAGTTCCGGTGTAGGTCGTTCCGTCCTCAA
15 GCTGGGCTGTGTGCAGAACCCCGCTCAGCCGACCGCTGCGCTTATCCGGTAACTA
TCGCTTTGAGTCAACCCCGTAAGACACGACTTATCGCCACTGCGCAGCAGCCACTGGTAA
CAGGATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTTCTTGAAGTGGTGGCTAA
CTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
CGGAAAAAGAGTTGGTAGCTCTTATCCGGCAAAACAAACCCGCTGGTAGCGGTGGTTT
20 TTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGAT
CTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACTCAGTTAAGGGATTTTGGTCAT
GAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAAAAATGAAGTTTAAATC
AATCTAAAGTATATAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGC
ACCTATCTCAGCGATCTGTCTATTTCTGTTATCCATAGTTGCTGACTCCCCGCTGTGA
25 GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGA
CCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCG
CAGAAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGC
TAGAGTAAGTAGTTCCGCAAGTAAAGTTTGGCGAACGTTGTTGCCATTGCTACAGGCAT
CGTGGTGTACGCTCGTCTGTTGGTATGGCTTCACTCAGCTCCGGTTCCCAACGATCAAG
30 GCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGTCTCTTCCGCTCTCCGAT
CGTTGTGAGAAGTAAGTTGGCGCAGTGTATCACTCATGGTTATGGCAGCACTGCATAA
TCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAA
GTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTCCCGGCGTCAATACGGGA
TAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCATCTGAAAAACGTTCTTCCGGG
35 GCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGC
ACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGG
AAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACT
CTTCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACAT
40 ATTTGAATGATTTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAAGT
GCCACCTAAATTGTAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTTGTAAAAATC
AGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAG
ACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAAACAAGATCCACTATTAAAGAACGTG
GACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCACTACGTGAACCA
TCACCCTAATCAAGTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCTAAA
45 GGGAGCCCCCTTAGAGCTTGACGGGGAAGCCAACCTGGCTTATCGAAATTAATACG
ACTCACTATAGG

5

PEsynGP (SEQ ID No 53)

TCAATATTGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTA
TTGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTATATTGGCTCATGTCC
10 AATATGACCGCCATGTTGGCATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGG
GTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCC
GCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCAT
AGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGC
CCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCTATTGACGTCAATGA
15 CGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCCTACTTG
GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACAC
CAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTGACGT
CAATGGGAGTTTGTGTTGGCACCAAAATCAACGGGACTTTCGAAGTGTGTAACAATG
CGATCGCCCGCCCGTTGACGCAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATA
20 AGCAGAGCTCGTTTAGTGAACCGTCAGATCACTAGAAGCTTTATTGCGGTAGTTTATCAC
AGTTAAATGCTAACGCAGTCAGTGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGT
GACTCTCTTAAGGTAGCCTTGCAGAAGTTGGTGTGAGGCACTGGGCAGGTAAAGTATCAA
GGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTGAGACAGAGAAGACT
CTTGCGTTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCAC
25 AGGTGTCCACTCCAGTTCAATTACAGCTCTTAAGGCTAGAGTACTTAATACGACTCACT
ATAGGCTAGAGAATTCGCCACCATGGGCGATCCCTCACCTGGTCCAAAGCCCTGAAGAA
ACTGGAAAAAGTCAACGTTTCAGGGTAGCCAAAAGCTTACCACAGGCAATTGCAACTGGGC
ATTGTCCCTGGTGGATCTTTCCACGACACTAATTTCTGTTAAGGAGAAAGATTGGCAACT
CAGAGACGTGATCCCCCTCTTGGAGGACGTGACCCAAAACATTGTCTGGGCAGGAGCGCGA
30 AGCTTTTCGAGCGCACCTGGTGGGCCATCAGCGCAGTCAAAATGGGGCTGCAAAATCAACAA
CGTGGTTGACGGTAAAGCTAGCTTTCAACTGTCTCCGCGCTAAGTACGAGAAGAAAACCGC
CAACAAGAAACAATCCGAACCTAGCGAGGAGTACCAATTATGATCGACGGCGCGCGGCA
TAGGAACTTCCGCCCACTGACTCCAGGGGCTATACCACCTGGGTCAACACCATCCAGAC
AAACGGGACTTTGAACGAAGCCTCCAGAACCTGTTCCGCATCCTGTCTGTGACTGCAC
35 CTCCGAAGAAATGAATGCTTTTCTCGACGTGGTGCCAGGACAGGCTGGACAGAAACAGAT
CCTGCTCGATGCCATTGACAAGATCGCCGACGACTGGGATAATCGCCACCCCTGCCAAA
CGCCCTCTGGTGGCTCCCCACAGGGGCTATCCCTATGACCGCTAGGTTTATTAGGGG
ACTGGGGGTGCCCCGCGAACGCCAGATGGAGCCAGCATTTGACCAATTTAGGCAGACCTA
CAGACAGTGGATCATCGAAGCATGAGCGAGGGGATTAAAGTCATGATCGGAAAGCCCAA
40 GGCACAGAACATCAGGCAGGGGGCCAAGGAACCATACCCTGAGTTTGTGACAGGCTTCT
GTCCAGATTAAATCCGAAGGCCACCTCAGGAGATCTCCAAGTTCTTGACAGACACACT
GACTATCCAAAATGCAATGAAGAGTGCAGAAACGCCATGAGGCACCTCAGACCTGAAGA
TACCTGGAGGAGAAAAATGTACGCATGTGCGGACATTGGCACTACCAAGCAAAAGATGAT
GCTGCTCGCCAAAGGCTCTGCAAAACCGGCTGGCTGGTCCATTCAAAGGAGGAGCACTGAA
45 GGGAGGTCCATTGAAAGCTGCACAAACATGTTATAATTGTGGGAAGCCAGGACATTTATC
TAGTCAATGTAGAGCACCTAAAGTCTGTTTTAAATGTAAACAGCCTGGACATTTCTCAA
GCAATGCGAAGTGTTCAAAAACGGGAAGCAAGGGGCTCAAGGGAGGCCCCAGAAACA
AACTTTCCCGATACAAACAGAAGAGTCAGCACAAACAAATCTGTTGTACAAGAGACTCTCA
GACTCAAAATCTGTACCCAGATCTGAGCGAAATAAAAAAGGAATACAATGTCAAGGAGAA
50 GGATCAAGTAGAGGATCTCAACCTGGACAGTTTGTGGGAGTAACATACAATCTCGAGAAG
AGGCCCACTACCATCGTCTGATCAATGACACCCCTCTTAATGTGCTGCTGGACACCGGA
GGCAGACACCGGCTTCTCACTACTGCTCACTATAACAGACTGAAATACAGAGGAAGGAAA
TACCAGGGCACAGGCATCATCGCGTTGGAGGCAACGTGAAACCTTTTCCACTCTGTG
ACCATCAAAAAAGAGGGGAGACACATTAAACCAGAAATGCTGGTGGCGACATCCCCGT
55 ACCATCCTTGGCAGAGACATTCTCCAGGACCTGGGCGCTAAACTCGTGTGGCACAACTG
TCTAAGGAAAATCAAGTTCCGCAAGATCGAGCTGAAAGAGGGGCAAAATGGGTCCAAAAATC
CCCCAGTGGCCCTGACCAAGAGAAGCTTGAGGGCGCTAAGGAAATCGTGCAGCGCCTG
CTTTCTGAGGGCAAGATTAGCGAGGCCAGCGACAATAACCCTTACAACAGCCCCATCTTT

GTGATTAAGAAAAGGAGCGGCAATGGAGACTCCTGCAGGACCTGAGGGAACTCAACAAG
ACCGTCCAGGTCGGAAGTGAATCTCTCGCGGACTGCCTCACCCCGCGGCCTGATTAAA
TGCAAGCACATGACAGTCCCTTGACATTGGAGACGCTTATTTTACCATCCCCCTCGATCCT
5 GAATTTGCGCCCTATACTGCTTTTACCATCCCCAGCATCAATCACCAGGAGCCCGATAAA
CGCTATGTTGGGAAGTGCTCCCCAGGGATTGTGCTTAGCCCTACATTTACCAGAAG
ACACTTCAAGAGATCCTCCAACCTTTCGCGAAAGATACCCAGAGGTTCAACTCTACCAA
TATATGGACGACCTGTTTCATGGGGTCCAACGGGTCTAAGAAGCAGCACAAGGAAGTCAATC
ATCGAAGTGAAGGCAATCCTCCTGGAGAAAGGCTTCGAGACACCCGACGACAAGCTGCAA
GAAGTTCCTCCATATAGCTGGCTGGCTACCAGCTTTGCCCTGAAAACTGGAAAGTCCAG
10 AAGATGCAGTTGGATATGGTCAAGAACCACACTGAACGACGTCCAGAAGCTCATGGGC
AATATTACCTGGATGAGCTCCGGAATCCCTGGGCTTACCGTTAAGCACATTGCCGCACT
ACAAAAGGATGCCTGGAGTTGAACCAGAAGGTCATTTGGACAGAGGAAGCTCAGAAGGAA
CTGGAGGAGAAATAATGAAAAGATTAAGAATGCTCAAGGGCTCCAATACTACATCCCAGAA
GAAGAAATGTTGTGCGAGGTGAAATCACTAAGAACTACGAAGCCACCTATGTCATCAAA
15 CAGTCCCAAGGCATCTTGTGGGCGGAAAGAAAATCATGAAGGCCAACAAAGGCTGGTCC
ACCGTTAAAAATCTGATGCTCCTGCTCCAGCAGTCGCCACCGAGTCTATCACCCGCGTC
GGCAAGTGGCCCACTTCAAAGTTCCCTTCACTAAGGAGCAGGTGATGTGGGAGATGCAA
AAAGGCTGGTACTACTCTTGGCTTCCCGAGATCGTCTACCCCACTAAGTGGTGCACGAC
GACTGGAGAAATGAAGCTTGTGAGGAGGCCACTAGCGGAATTACAATCTATACCGACGGC
20 GGAAAGCAAAACGGAGAGGGAATCGCTGCATACGTCACATCTAACGGCCGACCAAGCAA
AAGAGGCTCGGCCCTGTCACTCACCAGGTGCTGAGAGGATGGCTATCCAGATGGCCCTT
GAGGACACTAGAGACAAGCAGGTGAACATTGTGACTGACAGCTACTACTGCTGGAAAAAC
ATCACAGAGGGCCTTGGCCTGGAGGGACCCAGTCTCCCTGGTGGCCTATCATCCAGAAT
ATCCGCGAAAAGGAAATTTGCTATTTCCGCTGGGTGCTGGACACAAAGGAATTTACGGC
25 AACCAACTCGCCGATGAAGCCGCCAAAATTAAGAGGAAATCATGCTTGCCTACCAGGGC
ACACAGATTAAAGGAGAAGAGAGACGAGGACGCTGGCTTTGACCTGTGTGTGCCATACGAC
ATCATGATTTCCCGTTAGCGACACAAAGATCATTCCAACCGATGTCAAGATCCAGGTGCCA
CCCAATTCATTTGGTTGGGTGACCGGAAAGTCCAGCATGGCTAAGCAGGGTCTTCTGATT
AACGGGGGAATCATTGATGAAGGATACACCGCGAAATCCAGGTGATCTGCACAAATATC
30 GGCAAAAGCAATATTAAGCTTATCGAAGGGCAGAAAGTTCCGCTCAACTCATCTCTCCAG
CACCACAGCAATTAAGACAACCTTGGGACGAAAACAAGATTAGCCAGAGAGGTGACAAAG
GGCTTCGGCAGCACAGGTGTGTCTGGGTGGAGAATCCAGGAAGCACAGGACGAGCAC
GAGAATTGGCACACCTCCCTAAGATTTTGGCCGCAATTACAAGATCCCACTGACTGTG
GCTAAGATTAAGGACACACAGGAATGCCCCCACTGCACAAACAAGGTTCTGGCCCGCGGGC
35 TGCGTGATGAGGTCCCCAATCACTGGCAGGCAGATTGCACCCACCTCGACAACAAAATT
ATCCTGACCTTCGTGGAGAGCAATTCGGCTACATCCACGCAACACTCCTCTCCAAGGAA
AATGCATTGTGCACCTCCCTCGCAATTTCTGGAATGGGCCAGGCTGTTCTCTCCAAAATCC
CTGCACACCGACAACGGCACCAACTTTGTGGCTGAACCTGTGGTGAATCTGCTGAAGTTC
CTGAAAATCGCCACACCACTGGCATTCCCTATCACCTGAAAGCCAGGGCATTGTGCGAG
40 AGGGCCAACAGAACTCTGAAAGAAAAGATCCAATCTCACAGAGACAATACACAGACATTG
GAGGCCGCACTTCAGCTCGCCCTTATCACTGCAACAAAGGAAGAGAAAGCATGGGCGGC
CAGACCCCTGGGAGGTCTTCATCACTAACCAAGGCCAGGTGATCCATGAAAAGCTGCT
TTGACGAGGGCCAGTCTCTCAAAAAGTTCTGCTTTTATAAGATCCCGGTGAGCACGAC
TGGAAAGGTCTTACAAGAGTTTGTGGAAAGGAGACGGCGCAGTTGTGGTGAACGATGAG
45 GGCAAGGGGATCATCGCTGTGCCCTGACACGCACCAAGCTTCTCATCAAGCCAACTGA
ACCCGGGGCGGCCGCTTCCCTTTAGTGAGGTTAATGCTTCGAGCAGACATGATAAGATA
CATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAAATGCTTTATTTGTGA
AATTTGTGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAACAAAGTTAAACA
CAACAATTGCATTCAATTTATGTTTCAGGTTCAAGGGGAGATGTGGGAGGTTTTTAAAG
50 CAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGATCGATCCGGGCTGGCGTAAT
AGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGG
ACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGGCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTCTCGCCA
CGTTCGCGCGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTA
55 GAGCTTTACGGCACCTCGACCGCAAAAACTTGATTTGGGTGATGGTTACGTAGTGGGC
CATCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTG
GACTCTTGTTCAAAACCTGGAACAACACTCAACCTATCTCGGTCTATTCTTTGATTTAT
AAGGGATTTTGGCGATTTGGCCTATTGGTTAAAAAATGAGCTGATTTAACAATATTTA
ACGCGAATTTTAAACAAATATTAACGTTTACAATTTCCGCTGATGCGGTATTTTCTCCTT
60 ACGCATCTGTGCGGTATTTACACCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAA
TAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGAAAGAACAGCTGTGGA
ATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCAAA
GCATGCATCTCAATTAGTCAGCAACAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCA
GAAGTATGCAAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGC
65 CCATCCCGCCCTAACTCCGCCAGTTCGCCCATTTCTCGCCCATGGCTGACTAATTT
TTTTATTTATGCAGAGGCCGAGGCCGCTCGGCTCTGAGCTATTCCAGAAGTAGTGAG

GAGGCTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCT
CGAACTTAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGC
CGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGA
TGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCGGTTCTTTTTGTCAGACCGACCT
5 GTCCGGTGCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGAC
GGGCGTTCTTGGCAGCTGTGCTCGACGTTGTCACTGAAGCGGAAGGGACTGGCTGCT
ATTGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTACCTTGCTCCTGCCGAGAAAGT
ATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATT
CGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTA CTGGATGGAAGCCGGTCTTGT
10 CGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACGTTCGCCAG
GCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCT
GCCGAATCATGTGGTGGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGG
TGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGG
CGGCGAATGGGTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGACGG
15 CATCGCTTCTATCGCTTCTTGACGAGTCTTCTGAGCGGGACTCTGGGTTCCGAAATG
ACCGACCAAGCGACGCCAACCTGCCATCACGATGGCCGCAATAAAATATCTTTATTTTC
ATTACATCTGTGTGTTGTTTTGTGTGAATCGATAGCGATAAGGATCCGCGTATGGTG
CACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACCCGCCAAC
ACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGCATCCGCTTACAGACAAGCTGT
20 GACCGTCTCCGGAGCTGCATGTGTGACAGGTTTTACCGTCATCACCGAAACGCGCGAG
ACGAAAGGGCCTCGTGATACGCCTATTTTATAGGTTAATGTATGATAATAATGGTTTC
TTAGACGTCAGGTGGCACTTTTCGGGGAATGTGCGCGGAACCCCTATTTGTTTATTTT
CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATA
ATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTT
25 TGCGGCAATTTGCCCTTCTGTTTTGCTCACCCAGAAACGCTGGTGAAGTAAAAGATGC
TGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGAT
CCTTGAGAGTTTTGCCCGGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCT
ATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCCCGCATACA
CTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGG
30 CATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAA
CTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGACAACATGGG
GGATCATGTAACCTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGA
CGAGCGTGACACCAGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGG
CGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGCGGATAAAGT
35 TGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGG
AGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTC
CCGTATCGTAGTTATCTACACGAGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACGTGCAGACCAAGTTTACTC
ATATATACTTTAGATTGATTTAAAACTTCATTTTAAATTTAAAGGATCTAGGTGAAGAT
40 CCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTC
AGACCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGGTAATCTG
CTGCTTGCAAAACAAAAAACCCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCT
ACCAACTCTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAACTACTGTCT
TCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCCTACATACCT
45 CGCTCTGCTAATCTGTTACCACTGGCTGCCAGTGGCGATAAGTCTGTCTTACCGG
GTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGGTTC
GTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGTACCTACAGCGTGA
GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGG
CAGGGTCCGAACAGGAGAGCGCAGGAGGCTTCCAGGGGGAACGCTGGTATCTTTA
50 TAGTCTGTGCGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGG
GGGGCGGAGCTATGGAACCGCCAGCAACGCGGCTTTTTACGGTTCCTGGCCTTTTG
CTGGCCTTTTGCTACATGGCTCGACAGATCT

PESDSYNGP (SEQ ID No 54)

55 TCAATATTGGCCATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTA
TTGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATTGGCTCATGTCC
AATATGACCGCCATGTTGGCATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGG
GTCATTAGTTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCC
60 GCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCAT
AGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGC
CCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGA
CGGTAATGGCCCGCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCCTACTTG
GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACAC
65 CAATGGGCGTGATAGCGGTTTGACTACGGGGATTCCAAGTCTCCACCCATTGACGT

CAATGGGAGTTTGTGGTGGCACC AAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTG
CGATCGCCCGCCCCGTTGACGCAAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATA
AGCAGAGCTCGTTTAGTGAACCGTCAGATCACTAGAAGCTTTATTGCGGTAGTTTATCAC
AGTTAAATTGCTAACCGCAGTCAGTGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGT
5 GACTCTCTTAAGGTAGCCTTGCAGAAGTTGGTCGTGAGGCACTGGGCAGGTAAGTATCAA
GGTTACAAGACAGGTTTAAAGGAGACC.AATAGAACTGGGCTTGTGAGACAGAGAAGACT
CTTGGCTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCCTTCTCTCCAC
AGGTGTCCACTCCCGCTCAATTACAGCTCTTAAGGCTAGAGTACTTAATACGACTCACT
ATAGGCTAGAGAATTCCAGGTAAGATGGGCGATCCCTCACCTGGTCCAAAGCCCTGAAG
10 AAAGTGGAAAAAGTCACCGTTCAAGGTAGCCAAAAGCTTACCACAGGCAATTGCAACTGG
GCATTGTCCCTGGTGGATCTTTCCACGACACTAATTCGTTAAGGAGAAAAGATTGGCAA
CTCAGAGACTGTGATGCCCTCTTGGAGGACGTGACCCAAACATTGTCTGGGCAGGAGCGC
GAAGCTTTCGAGCGCACCTGGTGGGCCATCAGCGCAGTCAAAATGGGCTGCAAAATCAAC
AACGTGGTTGACGGTAAAGCTAGCTTTCAACTGCTCCGCGCTAAGTACGAGAAGAAAACC
15 GCCAACAGAAAACATCCGAACCTAGCGAGGAGTACCCAATTATGATCGACGGCGCCGGC
AATAGGAACCTCCGCCACTGACTCCAGGGGCTATACCACCTGGGTCAACACCATCCAG
ACAAACGGGACTTTTGAAACGAAGCCTCCAGAACCTGTTGGGCATCCTGTCTGTGGACTGC
ACCTCCGAAGAAATGAATGCTTTTCTCGACGTGGTGCCAGGACAGGCTGGACAGAAAACAG
ATCCTGCTCGATGCCATTGACAAGATCGCCGACGACTGGGATAATCGCCACCCCTGCCA
20 AACGCCCTGTGATGGCTCCCCACAGGGGCTATCCCTATGACCGCTAGGTTCTATTAGG
GGACTGGGGGTGCCCCGCGAACGCCAGATGGAGCCAGCATTGACCAATTTAGGCAGACC
TACAGACAGTGGATCATCGAAGCCATGAGCGAGGGGATTAAGTCAATGATCGGAAAGCCC
AAGGCACAGAACATCAGGCAGGGGGCCAAAGGAACCATACCCTGAGTTTGTGACAGGCTT
CTGTCCAGATTAATCCGAAGGCCACCCTCAGGAGATCTCCAAGTTCTTGACAGACACA
25 CTGACTATCCAAAATGCAAAATGAAGAGTGCAGAAACGCCATGAGGCACCTCAGACCTGAA
GATACCTGGAGGAGAAAATGTACGCATGTCGCGACATTGGCACTACCAAGCAAAAGATG
ATGCTGCTCGCCAAAGGCTCTGCAAAACCGGCTGGCTGGTCCATTCAAAGGAGGAGCACTG
AAGGGAGGTCCATTGAAAGCTGCACAAACATGTTATAATTGTGGGAAGCCAGGACATTTA
TCTAGTCAATGTAGACACCTAAAGTCTGTTTTAAATGTAAACAGCCTGGACATTTCTCA
30 AAGCAATGCAGAAAGTGTTCAAAAAACGGGAAGCAAGGGGCTCAAGGGAGGCCCCAGAAA
CAAATTTCCCGATACAACAGAAGAGTCAAGCACAACAAATCTGTTGTACAAGAGACTCCT
CAGACTCAAAATCTGTACCCAGATCTGAGCGAAATAAAAAAGGAATACAATGTCAAGGAG
AAGGATCAAGTAGAGGATCTCAACCTGGACAGTTTGTGGGAGTAACATACAATCTCGAGA
AGAGGCCCACTACCATCGTCTGATCAATGACACCCCTCTTAATGTGCTGCTGGACACCG
35 GAGCCGACACCAAGCGTTCTCACTACTGCTCACTATAACAGACTGAAATACAGAGGAAGGA
AATACCAGGGCACAGGCATCATCGGCGTTGGAGGCAACGTCGAAACCTTTTCACTCCTG
TCACCATCAAAAAGAGGGGAGACACATTAACCAAGAAATGCTGGTCCGCCGACATCCCCG
TCACCATCCTTGGCAGAGACATTCTCCAGGACCTGGGCGCTAAACTCGTGTGCTGGCACAAC
TGTCTAAGGAAATCAAGTTCCGCAAGATCGAGCTGAAAGAGGGCACAATGGGTCCAAAAA
40 TCCCCAGTGGCCCCGTGACCAAGAGAAAGCTTGAGGGCGCTAAGGAAATCGTGCAGCGCC
TGCTTTCTGAGGGCAAGATTAGCGAGGCCAGCGACAATAACCTTACAACAGCCCCATCT
TTGTGATTAAGAAAAGGAGCGGCAAAATGGAGACTCCTGCAGGACCTGAGGGAACCTCAACA
AGACCGTCCAGGTCCGAACTGAGATCTCTCGCGACTGCCTCACCCCGGCGGCCTGATTA
AATGCAAGCACATGACAGTCTTGACATTGGAGACGCTATTTTACCATCCCCCTCGATC
45 CTGAATTCGCCCTATACTGCTTTTACCATCCCCAGCATCAATACCAGGAGCCCGATA
AACGCTATGTGTGGAAAGTGCTTCCCCAGGGATTGTGCTTAGCCCCACATTTACCAGA
AGACACTTCAAGAGATCCTCAACCTTTCCGCGAAAGATACCCAGAGGTTCAACTTACC
AATATATGGACGACCTGTTATGCGGTCCAACGGGTCTAAGAAAGCAGCACAAGGAACCTCA
TCATCGAACTGAGGGCAATCCTCCTGGAGAAAGGCTTCGAGACACCCGACGACAAGCTGC
50 AAGAAGTTCCTCCATATAGCTGGCTGGGCTACCAGCTTTGCCCTGAAAACCTGGAAAGTCC
AGAAGATGCAGTTGGATATGGTCAAGAACCCAACTGAACGACGTCCAGAAGCTCATGG
GCAATATTACCTGGATGAGCTCCGGAATCCCTGGGCTTACCGTTAAGCACATTGCCGCAA
CTACAAAAGGATGCCTGGAGTTGAACAGAAGGTCAATTTGGACAGAGGAAGCTCAGAAGG
AAGTGGAGGAGAATAATGAAAAGATTAAAGATGCTCAAGGGCTCCAATACTACAATCCCCG
55 AAGAAGAAATGTTGTGCGAGGTGCAAAATCACTAAGAACTACGAAGCCACCTATGTCATCA
AACAGTCCCAAGGCATCTTGTGGGCGGAAAGAAAATCATGAAGGCCAACAAAGGCTGGT
CCACCGTTAAAAATCTGATGCTCCTGCTCCAGCACGTGCCACCGAGTCTATCACCCGCG
TCGGCAAGTGGCCCCACCTTCAAGTTCCCTTCACTAAGGAGCAGGTGATGTGGGAGATGC
AAAAAGGCTGGTACTACTCTTGGCTTCCCGAGATCGTCTACCCACCAAGTGGTGCACG
60 ACGACTGGAGAAATGAAGCTTGTGAGGAGGCCACTAGCGGAATTACAATCTATACCGACG
GCGGAAAGCAAAACGGAGAGGGAATCGCTGCATACGTCACTAATCAACGGCCGACCAAGC
AAAAGAGGCTCGGCCCTGTCACTACCAGGTGGCTGAGAGGATGGCTATCCAGATGGCCC
TTGAGGACACTAGAGACAAGCAGGTGAACATTGTGACTGACAGCTACTACTGCTGAAAAA
ACATCACAGAGGGCCTTGGCTGGAGGGACCCAGTCTCCTGGTGGCTATCATCCAGA
65 ATATCCGCGAAAAGGAAATGTCTATTTCGCTGGGTGGCTGGACACAAAGGAATTTACG
GCAACCAACTCGCCGATGAAGCCGCCAAAATTAAGAGGAAATCATGCTTGCTACCAGG

GCACACAGATTAAGGAGAAGAGAGACGAGGACGCTGGCTTTGACCTGTGTGTGCCATACG
 ACATCATGATTTCCCGTTAGCGACACAAAGATCATTCCAACCGATGTCAAGATCCAGGTGC
 CACCCAATTCATTTGGTTGGGTGACCGGAAAGTCCAGCATGGCTAAGCAGGGTCTTCTGA
 TTAACGGGGGAATCATTGATGAAGGATACACCGGCGAAATCCAGGTGATCTGCACAAATA
 5 TCGGCAAAAGCAATATTAAGCTTATCGAAGGGCAGAAAGTTCGCTCAACTCATCATCTCC
 AGCACACAGCAATTCAGACAACCTTGGGACGAAAACAAGATTAGCCAGAGAGGTGACAA
 AGGGCTTCGGCAGCACAGGTGTGTTCTGGGTGGAGAACATCCAGGAAGCACAGGACGAGC
 ACGAGAAATGGCACACCTCCCTAAGATTTTGGCCCGCAATTACAAGATCCCACTGACTG
 TGGCTAAGCAGATCACACAGGAATGCCCCACTGCACCAACAAGTTCTGGCCCCCGCG
 10 GCTGCGTGATGAGGTCCCCCACTGGCAGGCAGATTGCACCCACCTCGACAACAAAA
 TTATCTGACCTTCGTGGAGAGCAATTCGGGTACATCCACGCAACACTCCTCTCCAAGG
 AAAATGCATTGTGCACCTCCCTCGCAATTCTGGAATGGGCCAGGTGTCTCTCCAAAT
 CCCTGCACACCGACAACGGCACCAACTTTGTGGTGAACCTGTGGTGAATCTGCTGAAGT
 TCCTGAAAATCGCCACACCACTGGCATTCCCTATCACCTGAAAGCCAGGOCATTGTCTG
 15 AGAGGGCCAACAGAACTCTGAAAGAAAAGATCCAATCTCACAGAGACAATACACAGACAT
 TGGAGGGCCGCACTTACGCTCGCCCTTATCACTGCAACAAAGGAAGAGAAAGCATGGGGC
 GCCAGACCCCTGGGAGGTCTTCATCACTAACCAGGCCAGGTATCCATGAAAAGCTGC
 TCTGCAAGAGGGCCAGTCTCCAAAAAGTTCTGCTTTTATAAGATCCCCGGTGAGCAG
 ACTGGAAGGTCTACAAGAGTTTTGTGGAAGGAGACGGCGCAGTTGTGGTGAACGATG
 20 AGGGCAAGGGGATCATCGCTGTGCCCTGACACGCACCAAGCTTCTCATCAAGCCAACT
 GAACCCGGGGCGGCCGCTTCCCTTAGTGAGGGTTAATGCTTCGAGCAGACATGATAAGA
 TACATTGATGAGTTTGGACAAACCACTAGAATGCAGTGAAAAAATGCTTTATTGT
 GAAATTTGTGATGCTATTGCTTTATTGTAAACATTATAAGCTGCAATAAACAAGTTAAC
 AACAACAATTGCATTATTTATGTTTCAGGTTTCAGGGGAGATGTGGGAGGTTTTTAA
 25 AGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGATCGATCCGGGCTGGCGTA
 ATAGCGAAGAGGGCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAAT
 GGACGGCCCTGTAGCGGCGCATTAAAGCGCGGGGTGTGGTGGTTACGCGCAGCGTGAC
 CGCTACACTTGGCAGCGCCCTAGCGCCGCTCCTTTCGCTTCTTCCCTTCTTCTCGC
 CACGTTCCGCGGCTTTCCTCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTCCGATT
 30 TAGAGCTTACGGCACCTCGACCGCAAAAACTTGATTGGGTGATGGTTACGTAGTGG
 GCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAG
 TGGACTCTTGTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTGATTT
 ATAAGGGATTTTGCCGATTTCCGGCTATTGGTTAAAAAATGAGCTGATTTAACAATATT
 TAACGCGAATTTTAACAAAAATTAACGTTTACAATTCGCTGATGCGGTATTTTCTCC
 35 TTACGCACTGTGCGGTATTTACACCGCATACGCGGATCTGCGCAGCACCATGGCCTGA
 AATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCGGAAAGAACAGCTGTG
 GAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGAGAGTATGCA
 AAGCATGCATCTCAATTAGTCAGCAACCAAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAG
 CAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCTAACTCC
 40 GCCCATCCCCGCCCTAACTCCGCCAGTTCGCCCAATTCTCGCCCCATGGCTGACTAAT
 TTTTTTATTATGAGAGGCGCAGGCGCCCTCGGCCTCTGAGCTATTCCAGAAGTAGTG
 AGGAGGCTTTTTGGAGGCTAGGCTTTTGA AAAAGCTTGATTCTTCTGACACAACAGT
 CTCGAACCTAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGAGGTTCTCCG
 GCGGCTTGGGTGGAGAGGCTATTGGGCTATGACTGGGCACAACAGACAATCGGCTGCTCT
 45 GATGCCGCCGTGTTCCGGCTGTGAGCGAGGGGCGCCCGTTCTTTTGTCAAGACCGAC
 CTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCGAGCGGCTATCGTGGCTGGCCAG
 ACGGGCGTTCCTTGCGCAGCTGTGCTGACGTTGTCACTGAAGCGGGAAGGGACTGGCTG
 CTATTGGGCGAAGTGCCGGGGCAGGATCTCTGTCTACCTTGTCTCTGCCGAGAAA
 GTATCCATCATGGCTGATGCAATGCGGCGCTGCATACGCTTGATCCGGCTACCTGCCCA
 50 TTCGACCACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTT
 GTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACCTGTTCCGC
 AGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCTGCG
 TTGCCGAATATCATGGTGGAAAAATGGCCGCTTTTCTGGATTCTGACTGTGCCCGCTG
 GGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATTTGCTGAAGAGCTT
 55 GCGCGGAATGGGCTGACCGCTTCTCGTCTTACGGTATCGCGCTCCCGATTTCGAG
 CGCATCGCCTTCTATCGCTTCTTGACGAGTTCTTCTGAGCGGACTCTGGGTTTCAAAA
 TGACCGACCAAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATATCTTTATT
 TCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGATAAGGATCCGCGTATGG
 TGCATCTCAGTACAATCTGCTCTGATGCCGATAGTTAAGCCAGCCCCGACACCGCCA
 60 ACACCGCTGACCGGCCCTGACGGGCTTGTCTGCTCCGGCATCCGCTTACAGACAAGCT
 GTGACCGTCTCCGGGAGCTGCATGTGTGACAGGTTTTACCGTCTACCCGAAACGCGCG
 AGACGAAAGGGCCTCTGTATACGCTATTTTATAGGTTAATGTCTATGATAAATGGTT
 TCTTAGACGTCAGGTGGCACTTTTCCGGGAAATGTGCCGGAACCCCTATTTGTTTATT
 TTCTAAATACATTCAAAATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAA
 65 TAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCCGCTTATCCCTTT
 TTTGCGGCATTTGCTTCTGTTTTGCTCACCCAGAAACGCTGGTGAAGTAAAGAT

GCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAG
ATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTG
CTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGGCATA
CACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGAT
5 GGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCC
AACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATG
GGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAAC
GACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAAC
GGCGAACTACTTACTCTAGCTTCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAA
10 GTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCT
GGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCC
TCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGA
CAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTAC
TCATATATACTTTAGATTGATTTAAATCTCATTTTTAATTTAAAGGATCTAGGTGAAG
15 ATCCTTTTGTATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCG
TCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCTAATC
TGCTGCTTGCAAAACAAAAAACCCGCTACCAAGCGGTGGTTTGTGTTCCGGATCAAGAG
CTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTC
CTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATAC
20 CTCGCTCTGCTAATCCTGTTACAGTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACC
GGTTTGGACTCAAGACGATAGTTACCGGATAAGGGCGCAGCGGTGAGGCTGAACGGGGGT
TCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGT
GAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGC
GGCAGGGTCGGAACAGGAGAGCGCAGGAGGAGCTTCCAGGGGAAACGCCTGGTATCTT
25 TATAGTCCGTGCGGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCA
GGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTT
TGCTGGCCTTTTGCTCACATGGCTCGACAGATCT

MLV construct CZCG (SEQ ID No 55)

GTTACCTTCTGCTCTGCAGAAATGGCCAACTTTAACGTGGATGGCCGCGAGACGGCACC
TTTAACCGAGACCTCATCACCAGGTTAAGATCAAGGTCTTTTACCTGGCCCGCATGGA
CACCCAGACCAAGTCCCTTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCC
TGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCTCCTCTTCTCCATCCGCCCGCTCT
CTCCCCCTTGAACCTCCTCGTTCGACCCCGCTCGATCCTCCCTTTATCCAGCCCTCACT
35 CCTTCTCTAGGCGCCGGAATTCTGTTAACTCGAGAGGCTGCCACCATGGGGACTGCTCCA
AAGAAGAAGCGTAAGGTAGTCGTTTTACAACGTCTGACTGGGAAAACCTTGGCGTTACC
CAACTTAATCGCCTTGCAGCACATCCCCCTTTCCGCCAGCTGGCGTAATAGCGAAGAGGCC
CGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGG
TTTCCGGCACCAAGAGCGGTGCCGGAAGCTGGCTGGAGTGCGATCTTCTGAGGCCGAT
40 ACTGTCTGCTGCCCTCAAACCTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAAC
GTAACCTATCCATTACGGTCAATCCGCCGTTTGTTCACCGGAGAATCCGACGGGTTGT
TACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACCGCAATTATT
TTTGATGGCGTTAACTCGGCGTTTCACTGTGTTGCAACGGGCGCTGGGTGCGTTACGGC
CAGGACAGTCGTTTGGCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAAC
45 CGCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATG
TGGCGGATGAGCGGCATTTTCCGTGACGCTCGTTGCTGCATAAACCGACTACACAAATC
AGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTACGCCGCGCTGTACTGGAGGCT
GAAGTTTCAAGTGTGCGGCGAGTTGCGTGACTACCTACGGGTAAACAGTTTCTTTATGGCAG
GGTGAAACGCAGGTGCGCAGCGGCACCGCGCCTTTCCGGCGGTGAAATTATCGATGAGCGT
50 GGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTGAAACCCGAACTGTGGAGC
GCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCAGCTG
ATTGAAGCAGAGAGCCTGCGATGTCGGTTTTCCGCGAGGTGCGGATTGAAATGGTCTGCTG
CTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTG
CATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAAC
55 AACTTTAACGCCGTGCGCTGTTGCGATTATCCGAACCATCCGCTGTGGTACACGCTGTGC
GACCGCTACGGCCTGTATGTTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCA
ATGAATCGTGTACCGATGATCCGCGCTGGCTACCGCGATGAGCGAACGCGTAACCGCA
ATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTGGTGGGAATGAATCA
GGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATCTGTGATCCTTCCCGC

CCGGTGCAGTATGAAGGCGGCGAGCCGACACCACGGCCACCGATATTATTTGCCCGATG
TACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATCAAAAAA
TGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATG
GGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCSTTTA
5 CAGGGCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGC
AACCCGTGGTCCGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGT
ATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGGTGACGGAAGCAAAACAC
CAGCAGCAGTTTTTCCAGTTCGCTTTATCCGGGCAAAACCATCGAAGTGACCAGCGAATAC
CTGTTCCGCTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCG
10 CTGGCAAGCGGTGAAGTGCCCTCTGGATGTGCTCCACAAGGTAACAGTTGATTGAACTG
CCTGAACTACCGCAGCCGGAGAGCGCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAA
CCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGGCAGCAGTGGCGTCTG
GCGGAAAACCTCAGTGTGACGCTCCCCGCGCGTCCACGCCATCCCGCATCTGACCACC
AGCGAAATGGATTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCA
15 GGCTTCTTTTACAGATGTGGATTGGCGATAAAAAACAACCTGCTGACGCCGCTGCGCGAT
CAGTTACCCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGATTGAC
CCTAACGCGCTGGTTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTG
TTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTCAGCGTGG
CAGCATCAGGGGAAAACCTTATTTATCAGCCGAAAACCTACCAGATTGATGGTAGTGGT
20 CAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGCGCGGATT
GGCCTGAACTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAACTGGCTCGGATTAGGGCCG
CAAGAAAACCTATCCCGACCGCTTACTGCCGCTGTTTGGACCGCTGGGATCTGCCATTG
TCAGACATGTATACCCCGTACGCTCTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGC
GAATTGAATTATGGCCACACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTAC
25 AGTCAACAGCAACTGATGGAAACAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCACA
TGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCA
GTATCGGCGGAATTCCAGCTGAGCGCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAA
AAATAATAATAACCGGGCAGGGGGATCCGAGATCCGGCTGTGGAATGTGTGTCAGTTA
GGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCCCTGCAGGA
30 GTGGGGAGGCACGATGGCCGCTTGGTTCGAGGCGGATCCGGCCATTAGCCATATTATTCA
TTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGTATCCATATC
ATAATATGTACATTATATTGGCTCATGTCCAACATTACCGCATGTTGACATTGATTAT
TGACTAGTTATTAATAGTAATCAATTACGGGGTCAATTAGTTTCATAGCCCATATATGGAGT
TCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCC
35 CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC
GTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATA
TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCC
AGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA
TTACCATGGTGTATGCGGTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCAC
40 GGGGATTTCGAAGTCTCACCCCATTTGACGTCAATGGGAGTGTGTTTGGACCAAAATC
AACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAAATGGCGGTAGGC
ATGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGA
GACGCCATCCACGCTGTTTGGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCG
GCCCCAAGCTTGTGGGATCCACCGGTGCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTT
45 CACCGGGGTGGTGCCCATCTGGTTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAG
CGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTTCATCTG
CACCACCGCAAGCTGCCCGTGCCCTGGCCACCCCTCGTGACCACCTGACCTACGGCGT
GCAGTGCTTCAGCCGCTACCCCGACCATGAAGCAGCAGCACTTCTCAAGTCCGCCAT
GCCCCAAGGCTACGTCCAGGAGCGACCATCTTCTTCAAGGACGACGGCAACTACAAGAC
50 CCGCGCCGAGGTGAAGTTCGAGGGCGACACCTGGTGAACCGCATCGAGCTGAAGGGCAT
CGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACATAACAGCCA
CAACGCTTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAGTTCAAGATCCG
CCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCAT
CGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCAGTCCGCCCCGAG
55 CAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCCGCGG
GATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGATCATAATC
AGCCATACCACATTTGTAGAGGTTTACTTGCTTTAAAAACCTCCACACCTCCCCCTG
AACCTGAACATAAAATGAATGCAATTGTTGTTGTTAACATCGATAAAATAAAGATTTT
ATTTAGTCTCCAGAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGC
60 TTAAGTAACGCCATTTTGAAGGCATGAAAAATACATAACTGAGAATAGAGAAGTTCAG

ATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAG
CAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAG
GATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGAT
CGGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTCCAGGGTGCCCCAAGGA
5 CCTGAAATGACCCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTC
GCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCACAACCCCTCACTCGGGGCGCCAG
TCCTCCGATTGACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAATTGC
ATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGT
CAGCGGGGTCTTTCAATTTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCAGGGACCA
10 CCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTATCTGTGTCTGTCCGATTGTCTA
GTGCTATGACTGATTTTATGCGCCTGCGTCCGTACTAGTTAGCTAACTAGCTCTGTATC
TGGCGGACCCGTGGTGGAACTGACGAGTTCGGAACACCCGGCCGCAACCCCTGGGAGAGGA
ATTCTCATGTTTGACAGCTTATCATCGATAAGCTTTTGGCAAAGCCTAGGCCCTCCAAAA
AAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCCTCGGCCTCTGCATAA
15 ATAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAAGTGGGCGGAGTTAGGGGC
GGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGC
ATACTTCTGCGCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGA
TGCATGCTTTGCATACTTCTGCGCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAAGTGA
CACACATTCCACAGCCGGATCCTCTACGCCGGACGCATCGTGGCCGGCATACCCGGCGCC
20 ACAGGTGCGGTGTCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGC
CACTTCGGGCTCATGAGCGCTTGTTCGGCGTGGGTATGGTGGCAGGCCCGTGGCCGGG
GGACTGTTGGGCGCCATCTCCTGCAATGACCAATCCTTGGCGCGGCGGTGCTCAACGGC
CTCAACCTACTACTGGGCTGCTTCTCTAATGCAGGAGTCGATAAGGGAGAGCGTCGACCG
ATGCCCTTGAGAGCCTTCAACCCAGTCAGCTCCTTCGGTGGGCGCGGGGCATGACTATC
25 GTCGCCGCACCTATGACTGTCTTCTTTATCATGCAACTCGTAGGACAGGTGCCGGCAGCG
CTCTGGGTCAATTTTCGGCGAGGACCGCTTTCGCTGGAGCGCGACGATGATCGGCCTGTCTG
CTTGCGGTATTCGGAATCTTGACGCCCCCTCGCTCAAGCCTTCGTCACTGGTCCCGCCACC
AAACGTTTCGGCGAGAAGCAGGCCATTATCGCCGGCATGGCGGCGGACGCGCTGGGCTAC
GTCTTGCTGGCGTTTCGCGACGCGAGGCTGGATGGCCTTCCCATATGATTCTTCTCGCT
30 TCCGGCGGCATCGGGATGCCCGGTGCGAGGCTGCTGTCCAGGCAGGTAGATGACGAC
CATCAGGGACAGCTTCAAGGATCGCTCGCGGCTCTTACCAGCCTAACTTCGATCACTGGA
CCGCTGATCGTCACGGCGATTATGCGCCCTCGGCGAGCACATGGAACGGGTGGCATGG
ATTGTAGGCGCCGCCCTATACCTTGTCTGCCTCCCCGCGTTGCGTGGCGGTGCATGGAGC
CGGGCCACCTCGACCTGAATGGAAGCCGGCGGCACCTCGCTAACGGATTACCACTCCAA
35 GAATGGAGCCCAATCAATTCCTTGGCGAGAAGTGTGAATGCGCAAAACCCCTGGCAGA
ACATATCCATCGCGTCCGCCATCTCCAGCAGCCGACGCGGCGCATCTCGGGCAGCGTTG
GGTCTTGGCCACGGGTGCGCATGATCGTGTCTTGTGCTTGGAGACCCGGCTAGGCTGGC
GGGGTTGCCTTACTGGTTAGCAGAATGAATCACCGATACGCGAGCGAAGCTGAAGCGACT
GCTGTGCAAAACGCTGCGACCTGAGCAACAACATGAATGGTCTTCGGTTTCCGTGTTT
40 CGTAAAGTCTGGAACGCGGAAGTCAGCGCCCTGCACCATATGTTCCGGATCTGCATCG
CAGGATGCTGCTGGCTACCTGTGGAAACCTACATCTGTATTAAACGAAGCGCTGGCATT
GACCCGTAGTGATTTTCTCTGGTCCCGCCGATCCATACCGCCAGTTGTTTACCCTCAC
AACGTTCCAGTAACCGGGCATGTTTCATCATCAGTAACCCGTATCGTGAGCATCTCTCTC
GTTTCATCGGTATCATTACCCCCATGAACAGAAATCCCCCTTACACGGAGGCATCAAGT
45 GACCAAAACAGGAAAAACCGCCCTTAACATGGCCCGCTTATCAGAAGCCAGACATTAAC
GCTTCTGGAGAACTCAACGAGCTGGACGCGGATGAACAGGCAGACATCTGTGAATCGCT
TCACGACCAAGCTGTAGAGCTTTACCGCAGCTGCCTCGCGCTTTCGGTGATGACGGTGA
AAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGG
GAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCGCAGCCAT
50 GACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAAGTATCGGGCATCAGAGCAG
ATTGTACTGAGAGTGACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAA
TACCGCATCAGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTCTGCTCGG
CTGCGGCGAGCGGTATCAGCTCACTCAAAGCGGTAATACGGTTATCCACAGAATCAGGG
GATAACGCAAGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAG
55 GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA
CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACAGGCGTTTCCCCCT
GGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCC
TTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTTCG
GTGTAGTTCGTTTCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTTCAGCCCGACCGC
60 TCGCCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA

CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG
TTCTTGAAGTGGTGGCCTAACACGGCTACACTAGAAGGACAGTATTTGGTATCTGCCGT
CTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACC
ACCGCTGGTAGCGGTGGTTTTTTTGGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGA
5 TCTCAAGAAGATCCTTTTATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCA
CGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAAT
TAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTAC
CAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTT
GCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGT
10 GCTGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAGATTATCAGCAATAAACCCAG
CCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTTCAACTTTATCCGCCCTCCATCCAGTCT
ATTAATTTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTT
GTTGCCATTGCTGCAGGCATCGTGGTGTACGCTCGTCTTGGTATGGCTTCATTACGC
TCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTT
15 AGCTCCTTCGGTCTCCGATCGTCTGTGTCAGAACTAAGTTGGCCGAGTGTATCACTC
ATGGTTATGGCAGCACTGCATAATCTCTTACTGTATGCCATCCGTAAGATGCTTTTCT
GTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGC
TCTTGCCCGCGCTCAATACGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGCTC
ATCATTGGAACCGTTCTTCGGGGCGAAACTCTCAAGGATCTTACCGCTGTTGAGATCC
20 AGTTTCGATGTAAACCACTCGTGCACCAACTGATCTTCAGCATCTTTTACTTTACCCAGC
GTTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACA
CGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGT
TATTGCTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTT
CCGCGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACA
25 TTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTCGTCTCGCGCGTTTCGGTGATGAC
GGTGAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGG
CTTAACCTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATCGACGCTCTCCCT
TATGCGACTCCTGTCATTAGGAAGCAGCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCG
30 CCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCGGCCACGGGGCTG
CCACCATACCCACGCCGAAACAAGCGCTCATGAGCCGAAGTGCGAGCCGATCTTCCC
CATCCGTGATGTCGGCGATATAGGCGCCAGCAACCCGACCTGTGGCGCCGTTGATGCCGG
CCACGATGCGTCCGGCGTAGAGGATCTGGCTAGCGATGACCTGCTGATTGGTTCGCTGA
CCATTTCCGGGGTGGCGAACGGCGTTACCAGAACTCAGAAGGTTTCGTCCAACCAACCG
35 ACTCTGACGGCAGTTTACGAGAGAGATGATAGGGTCTGCTTCAGTAAGCCAGATGCTACA
CAATTAGGCTTGATACATATTGTCGTTAGAACGCGGCTACAATTAATACATAACCTTATGT
ATCATAACATACGATTTAGGTGACACTATAGAATAACAAGCTGGAAGATCTTCCAGCTTG
GGCTCAGGTCGACTCTAGAGTCCGTTACATAACTACGGTAATGGCCCGCTGGCTGA
CCGCCAACGACCCCGCCCATGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA
40 ATAGGGCACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCA
GTACATCACTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG
CCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATC
TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACATCAATGGGCGT
GGATAGCGGTTTGAATCAGCGGGATTTCCAAGTCTCCACCCCATGACGTCAATGGGAGT
45 TTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCATTG
ACGCAAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTG
AACCGCGCCAGTCTTCCGATAGACTGCGTCCGCCGGGTACCCGTATTCCCAATAAAGCCT
CTTGCTGTTTGCATCCGAATCGTGGTCTCGCTGTTCTTGGGAGGGTCTCCTCTGAGTGA
TTGACTACCCACGACGGGGTCTTTCAATTGGGGGCTCGTCCGGGATTGGAGACCCCTG
50 CCCAGGGACACCGACCCACCGGGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGT
CCGATTGTCTAGTGTCTATGTTGATGTTATGCGCTGCGTCTGTACTAGTTAGCTAACT
AGCTCTGTATCTGGCGGACCCGTGGTGGAACTGACGAGTTCTGAACACCCGGCCGCAACC
CTGGGAGAGCTCCAGGGACTTTGGGGGCGGTTTTTGTGGCCGACCTGAGGAAGGGAGT
CGATGTGGAATCCGACCCCGTCAGGATATGTGGTCTGGTAGGAGACGAGAACCTAAAC
55 AGTTCCCGCTCCGTCTGAATTTTGTCTTCGGTTTGGAAACCGAAGCCGCGCGTCTTGT
TGCTGAGCGCTGAGCATCGTCTGTGTGTCTGTCTGCTGACTGTGTTTCTGTATTTGT
CTGAAAATTAGGGCCAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGCTCACTGGAAG
ATGTCGAGCGGATCGCTCACAACCAAGTCCGTAGATGTCAAGAAGAGACGTTGG

GTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGGCACC
TTTAACCGAGACCTCATCACCAGGTTAAGATCAAGGTCTTTTCACCTGGCCCGCATGGA
CACCAGACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCC
5 TGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCTCCTCTTCTCCATCCGCCCCGTCT
CTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACT
CCTTCTCTAGGCGCCGGAATTCTGTTAACTCGAGAGGCCTGCCACCATGGGGACTGCTCCA
AAGAAGAAGCGTAAGGTAGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACC
CAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCC
10 CGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCTTG
TTCCGGCACCAGAAGCGGTGCCGGAAGCTGGCTGGAGTGGCATCTTCTGAGGCCGAT
ACTGTCGTCTGCTCCCTCAAACCTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAAC
GTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCACCGAGAATCCGACGGGTGT
TACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGGAATTATT
15 TTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTGCGTTACGGC
CAGGACAGTCGTTTGGCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAAC
CGCCTCGCGGTGATGGTGCTGCGTTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATG
TGGCGGATGACGGGCATTTTCCGTGACGTCGTTGCTGCATAAACCGACTACACAAATC
AGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTACGCGCGCTGTACTGGAGGCT
20 GAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAG
GGTGAACGCAGGTCGCCAGCGGCACCGCGCTTTCCGGCGGTGAAATTATCGATGAGCGT
GGTGGTTATGCCGATCGCGTCACACTACGTCGAACGTCGAAAACCCGAAACTGTGGAGC
GCCGAATCCCGAATCTCTATCGTGCGGTGGTTGAATGCACACCGCCGACGGCAGCGCTG
ATTGAAGCAGAAGCCTGCGATGTGCGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTG
25 CTGCTGAACGGCAAGCCGTTGCTGATTTCGAGGCGTTAACCGTCACGAGCATCATCCTCTG
CATGGTCAGGTCATGGATGAGCAGACGATGGTGACGATATCCTGCTGATGAAGCAGAAC
AACTTTAACGCCGTGCGCTGTTTCGATTATCCGAACCATCCGCTGTGGTACACGCTGTGC
GACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACGGCATGGTGCCA
ATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGCGATGAGCGAACCGGTAAACGCGA
30 ATGGTGACGCGCATCGTAATACCCGAGTGTGATCATCTGGTCGCTGGGAATGAATCA
GGCAACGGCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCTGGCAGCATGGCGTCTG
CCGGTGACGATGAAGGCGGCGGAGCCGACACCGCCACCGATATTATTGCCCCGATG
TACGCGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAATGGTCCATCAAAAAA
TGGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTGCGAATACGCCACCGCATG
35 GGTAACAGTCTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTGATATCCCCGTTA
CAGGCGCGCTTCGCTCGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGC
AACCCGTGGTCCGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCAGTTCTGT
ATGAACGGTCTGGTCTTTGCCGACCGCACGCGCATCCAGCGCTGACGGAAGCAAAACAC
CAGCAGCAGTTTTTCCAGTTCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAATAC
40 CTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGGCGCTGGATGGTAAGCCG
CTGGCAAGCGGTGAAGTGCTCTGGATGTGCTCCACAAGGTAAACAGTTGATTGAAGT
CCTGAATACCGCAGCCGAGAGCGCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAA
CCGAACCGGACCGCATGGTCAGAAGCCGGGCACATCAGCGCTGGCAGCATGGCGTCTG
GCGGAAAACCTCAGTGTGACGCTCCCCGCGCGTCCACGCCATCCCGCATCTGACCACC
45 AGCGAAATGGATTTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCA
GGCTTTCTTTCACAGATGTGGATTGGCGATAAAAAACAATGCTGACGCCGCTGCGCGAT
CAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCGCATTGAC
CCTAACCCCTGGGTGCAACGCTGGAAGGCGGCGGCCATTACCAGGCCGAAGCAGCGTTG
TTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTCAGCGTGG
50 CAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAACCTACCGGATTGATGGTAGTGGT
CAAATGGCGATTACCGTTGATGTTGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATT
GGCCTGAATGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCG
CAAGAAAATATCCCGACCGCCTTACTGCCGCTGTTTTGACCGCTGGGATCTGCCATTG
TCAGACATGTATACCCCGTACGCTTCCCGAGCGAAAACGGTCTGCGCTGCGGACGCGC
55 GAATTGAATTATGGCCACACCACTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTAC
AGTCAACAGCAACTGATGGAACACGACCATCGCCATCTGCTGCACGCGGAAGAAGGCACA
TGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGCCCGTCA
GTATCGGCGGAATTCCAGCTGAGCGCGGTGCTACCATACCAGTTGGTCTGGTGTCAA
AAATAATAATAACCGGGCAGGGGGATCCGCAGATCCGGCTGTGGAATGTGTGTAGTTA
60 GGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCCTGCAGGA

GTGGGGAGGCACGATGGCCGCTTTGGTCGAGGCGGATCCGGCCATTAGCCATATTATTCA
TTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGTATCCATATC
ATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTAT
TGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCCATATATGGAGT
5 TCCGCGTTACATAACTTACGGTAAATGGCCCCCTGGCTGACCGCCCCAACGACCCCCGCC
CATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGAC
GTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATA
TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGCCC
AGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA
10 TTACCATGGTGATGCGGTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCAC
GGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTGGCACCAAAATC
AACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAAATGGGCGGTAGGC
ATGTACGTTGGGAGGTCTATATAAGCAGAGCTCGTTAGTGAACCGTCAGATCGCCTGGA
GACGCCATCCAGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCG
15 GCCCCAAGCTTGTGGGATCCACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTCCCATCTGGTTCGAGCTGGACGGCGACGTAACCGGCCACAAGTTCAG
CGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTG
CACCACCGGCAAGCTGCCGTGCCCTGGCCACCCCTCGTGACCACCTGACCTACGGCGT
GCAGTGTCTCAGCCGCTACCCCGACCATGAAGCAGCAGCACTTCTTCAAGTCCGCCAT
20 GCCCGAAGGCTACGTCCAGGAGCGACCATCTTCTTCAAGGACGACGGCAACTACAAGAC
CCGCGCCGAGGTGAAGTTCGAGGGCGACACCTGGTGAACCGCATCGAGCTGAAGGGCAT
CGACTTCAAGGAGGACGGCAACATCCTGGGCGACAAGCTGGAGTACAACAGCCCA
CAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAGTTCAGATCCG
CCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCAT
25 CGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCAGCCAGTCCGCCCTGAG
CAAAGACCCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCGG
GATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGCCGCGACTCTAGATCATAATC
AGCCATACCACATTTGTAGAGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTG
AACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACATCGATAAAATAAAGATTTT
30 ATTTAGTCTCCAGAAAAAGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGC
ATACTTCGTATAATGTATGCTATACGAAGTTATTCTAGAGAACCATCAGATGTTTCCAG
GGTGCCCCAAGGACCTGAAATGACCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTT
CTCGCTTCTGTTCCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCACAACCCCTCA
CTCGGGGCGCCAGTCTCCGATTGACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACC
35 CTCTTGCAAGTTGCATCCGACTTGTGGTCTCGCTGTTCCCTGGGAGGGTCTCCTCTGAGTG
ATTGACTACCCGTGACGCGGGGTCTTTCAATTGGGGGCTCGTCCGGGATCGGGAGACCCC
TGCCCAGGGACCAACCGACCCACCGGGAGGTAAGCTGGCTGCCTCGCGCGTTTCGGTG
ATGACGGTGAACCACTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAG
CGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGGTGACGGGTGTTGGCGGGTGTCCGGG
40 GCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAACATGCGGGC
ATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGT
AAGGAGAAAAATACCGCATCAGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTCGCTC
GGTCTTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCAC
AGAATCAGGGGATAACGCAGGAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAA
45 CCGTAAAAAGGCCGCTTGTGCGGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCA
CAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACAGGC
GTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATA
CCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTA
TCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTCA
50 GCGGACCGCTGCGCCTTATCCGTAACATCGTCTTGTAGTCCAACCCGGTAAGACACGA
CTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGG
TGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGGACAGTATTTGG
TATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGG
CAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAG
55 AAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAA
CGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGAT
CCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTC
TGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTT
ATCCATAGTTGCCTGACTCCCCGTGCTGTAGATAACTACGATACGGGAGGGCTTACCATC
60 TGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAGATTTATCAGC

AATAAACCCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTC
CATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTT
GCGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTACGCTCGTTCGTTTGGTATGGC
TTCATTAGCTCCGGTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAA
5 AAAAGCGGTTAGTCTCTTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGT
ATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTGTCATGCCATCCGTAAGATG
CTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACC
GAGTTGCTCTTGGCCGGCGTCAACACGGGATAATACCGGCCACATAGCAGAAGTTTAAA
AGTGCTCATCATTGGAAAACGTCTCTCGGGCGAAAACCTCTCAAGGATCTTACCCTGT
10 GAGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTTACTTT
CACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAATGCCGCAAAAAGGGAATAAG
GGCGACACGAAATGTTGAATACTCATCTCTCTTTTCAATATTATTGAAGCATTTA
TCAGGGTTATTGTCTCATGAGCGGATACATATTGAATGTATTTAGAAAAATAAACAAAT
AGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCTGACGCTTAAGAAACCATTTATAT
15 CATGACATTAACCTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCAAGAATTCAT
ACCAGATCACGAAAACGTCTCTCAAATGTGTCCCCCTCACACTCCCAAATTCGCGGGC
TTCTGCCTCTTAGACCACTCTACCCTATTCCCCACACTCACCGGAGCCAAAGCGCGGCC
CTTCCGTTTCTTTGCTTTTGAAGACCCACCCGTTAGGTGGCAAGCTAGCGATGACCCTG
CTGATTGGTTCGCTGACCATTTCGCGGGTGCGGAACGGCGTTACCAGAACTCAGAAGGT
20 TCGTCCAACCAACCGACTCTGACGGCAGTTACGAGAGAGATGATAGGGTCTGCTTCAG
TAAGCCAGATGCTACACAATTAGGCTTGTACATATTGTCGTTAGAAGCGGGCTACAATTA
ATACATAACCTTATGTATCATACACATACGATTAGGTGACACTATAGAATACAAGCTGG
AAGATCTTCCAGCTTGGGCTGCAGGTGACTCTAGAGTCCGTTACATAACTTACGGTAAA
TGGCCCGCCTGGCTGACCGCCACGACCCCGCCCATGACGTCAATAATGACGTATGT
25 TCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTA
AACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGT
CAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCC
TACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCA
GTACATCAATGGGCGTGATAGCGGTTTGAATCACGGGATTTCCAAGTCTCCACCCCAT
30 TGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTCCAAAATGTCGTAA
CAACTCCGCCCCATTGACGCAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAG
CAGAGCTCGTTTAGTGAACCGCGCCAGTCTTCCGATAGACTGCGTCGCCCGGGTACCCGT
ATTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGGTCTCGCTGTTCTTGGGAG
GGTCTCTCTGAGTGATTGACTACCCACGACGGGGTCTTTCAATTTGGGGGCTCGTCCGG
35 GATTTGGAGACCCCTGCCAGGGACACCGACCCACCACCGGGAGGTAAGCTGGCCAGCA
ACTTATCTGTGTCTGTCGATTGTCTAGTGTCTATGTTGATGTTATGCGCCTCGCTCTG
TACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAAGTACGAGTCTGA
ACACCCGGCCGCAACCCTGGGAGACGTCCAGGGACTTTGGGGGCGGTTTGTGCCCCG
ACCTGAGGAAGGGAGTCGATGTGGAATCCGACCCCGCTCAGGATATGTGGTCTGGTAGGA
40 GACGAGAACCTAAACAGTTCCCGCTCCGTCTGAATTTTGTCTTTCGGTTTGGAAACCGA
AGCCGCGCGTCTTGTCTGCTGCAGCGCTGCAGCATCGTTCTGTGTTGTCTGTCTGACT
GTGTTTCTGTATTTGTCTGAAAATTAGGGCCAGACTGTTACCACTCCCTTAAGTTTGACC
TTAGGTCACTGGAAAGATGTGAGCGGATCGCTCACAACCAAGTCCGTAGATGTCAAGAAG
AGACGTTGG

45

PCGCLNGFR (SEQ ID No 57)

GTTACCTTCTGCTCTGCAGAATGGCCAACCTTAAAGTTCGGATGGCCGCGAGACGGCACC
TTTAAACCGAGACCTCATACCCAGGTTAAGATCAAGGTCTTTACCTGGCCCGCATGGA
50 CACCCAGACCAAGTCCCTTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCTCCC
TGGGTCAAGCCCTTTGTACACCTAAGCCTCCGCTCTCTTCTCCATCCGCCCCGTCT
CTCCCCCTTGAACCTCCTCGTTCCGACCCCGCTCGATCCTCCCTTTATCCAGCCCTCACT
CCTTCTCTAGGCGCCGGAATTCTGTTAACTCGAGGATCCACCGGTCCGACCATGGTGAGC
AAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTA
55 AACGGCCACAAGTTACGCGTGTCCGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTG
ACCCTGAAGTTATCTGCACACCGGCAAGCTGCCCGTGGCCACCCCTCGTGACC
ACCCTGACCTACGGCGTGAGTGTCTCAGCCGCTACCCGACCATGAAGCAGCAGAC
TTCTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGAC
GACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCGC
60 ATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG

TACAAC TACAACAGCCACAACGCTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAG
GTGAAC TTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTAC
CAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCCTACCTGAGC
ACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTCTGCTGGAG
5 TTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCCT
AGGGGTCTTTCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCA
GTTCTCTGGAAGCTTCTTGAAGACAAACAACGCTCTGTAGCGACCCCTTTCAGGCAGCGG
AACCCCCACCTGGCGACAGGTGCTCTGCGGCCAAAAGCCACCGAGTTGGTTTCACTGCT
TGCCTGAGGCTGGACGACCTCGCGGAGTTCTACCGGCAGTGCAATCCGTCCGCATCCAG
10 GAAACCAGCAGCGGCTATCCGCGCATCCATGCCCCGAACTGCAGGAGTGGGGAGGCAG
ATGGCCGCTTTGGTCGAGGCGGATCCGGCCATTAGCCATATTATTCATTGGTTATATAGC
ATAAATCAATATTGGCTATTGGCCATTGCATACGTTGTATCCATATCATAATATGTACAT
TTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTAGTTATTA
ATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCGTTACATA
15 ACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTGACGTCAAT
AATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGA
GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGATCATATGCCAAGTACGCC
CCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTT
ATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGAT
20 GCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAG
TCTCCACCCCATTGACGTCAATGGGAGTTTGTGTTGGCACCAAAATCAACGGGACTTTC
AAAATGTCGTAAACAACCTCGCCCATTGACGCAATGGGCGGTAGGCATGTACGGTGGGA
GGTCTATATAAGCAGAGCTCGTTTTAGTGAACCGTCAGATCGCTGGAGACGCCATCCAG
CTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCCAAGCTTAC
25 CATGGGGGCGAGTGCCACCGGCCCGCCATGGACGGGCGCGCTGCTGCTGTTGCTGCT
TCTGGGGGTGTCCTTGGAGGTGCCAAGGAGGCATGCCCCACAGGCCTGTACACACACAG
CGGTGAGTGCTGCAAAGCCTGCAACCTGGGCGAGGGTGTGGCCAGCCTGTGAGGCAAC
CCAGACCGTGTGTAGCCCTGCCTGGACAGCGTGACGTTCTCCGACGTGGTGGAGCGGAC
CGAGCCGTGCAAGCCGTGCACCGAGTGCCTGGGGCTCCAGAGCATGTCGGCGCGGTGCGT
30 GGAGGCCGACGACGCCGTGTGCCGTGCGCCTACGGCTACTACCAGGATGAGACGACTGG
GCGCTGCGAGGCGTGCCTGCGTGTGCGAGGCGGGCTCGGGCCTCGTGTTCTCTGCCAGGA
CAAGCAGAACACCGTGTGCGAGGAGTGCCCCGACGGCACGTATTCCGACGAGGCCAACCA
CGTGGAACCGTGCCTGCCCTGCACCGTGTGCGAGGACACCGAGCGCCAGCTCCGCGAGTG
CACACGCTGGGCCGACGCGGAGTGCAGGAGATCCCTGGCCGTGGATTACACGGTCCAC
35 ACCCCAGAGGGCTCGGACAGCACAGCCCCAGCACCCAGGAGCTGAGGCACCTCCAGA
ACAAGACCTCATAGCCAGCACGGTGGCAGGTGTGGTGACCACAGTGATGGGCAGCTCCCA
GCCCCTGGTGACCCGAGGCACACCGACAACCTCATCCCTGTCTATTGCTCCATCCTGGC
TGCTGTGGTTGTGGGCCCTTGTGGCCTACATAGCCTTCAAGAGGTGGAACAGCTGCTGAGT
CGACTCTAGAGGATCCCCAACATCGATAAAATAAAAGATTTTATTAGTCTCCAGAAAAA
40 GGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTG
CAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTTCAATCAAGGTGAGGAACAGA
TGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTTCCCCGGCTC
AGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAG
TTCTTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCA
45 GTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGC
CTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCG
AGCTCAATAAAAGAGCCCAACCCCTCACTCGGGGCGCCAGTCTCCGATTGACTGAGT
CGCCCGGGTACCCGTGTATCCAATAAACCCCTCTGTCAGTTGCATCCGACTTGTGGTCTCG
50 TGGGGGCTCGTCCGGATCGGGAGACCCCTGCCAGGGACACCGACCCACCGGGGAG
GTAAGCTGGCTGCCTCGCGGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCT
CCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGG
CGCGTCAGCGGGTGTGGCGGGTGTGCGGGCGCAGCCATGACCCAGTCACGTAGCGATAG
CGGAGTGTATACTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGAGAGTGACCAT
55 ATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCC
GTCTCCTCGCTCACTGACTCGCTGCGCTCGTTCGGTTCGGCTGCGGCGAGCGGTATCAGCT
CACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATG
TGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGCCCGTGTGCTGGCGTTTTTC
CATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGA
60 AACCCGACAGGACTATAAGATACCAGGCGTTTCCCTTGAAGCTCCCTCGTGGCTCT

CTGTTCGGACCCGCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTG
GCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAG
CTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACTAT
CGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAC
5 AGGATTAGCAGAGCGAGGTATGTAGCGGTGTACAGAGTCTTGAAGTGGTGGCCTAAC
TACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTC
GGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCCAGAAAAAAGGATCTCAAGAAGATCCTTTGATC
TTTTCTACGGGCTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTCATG
10 AGATTATCAAAAAGGATCTTCACCTAGATCCTTTAAATTAAAAATGAAGTTTTAAATCA
ATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCA
CCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAG
ATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAGAC
CCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGC
15 AGAAGTGGTCTGCACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCT
AGAGTAAGTAGTTCGCCAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTGCAGGCATC
GTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGG
CGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCCGATC
GTTGTCAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAAT
20 TCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAG
TCATTCTGAGAATAGTGTATGCGGCGACCGAGTGTCTTGGCCGGCTCAATACGGGAT
AATACCGCGCCACATAGCAGAACTTTAAAAGTGTCTCATCTGGAAAACGTTCTTCGGGG
CGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCACTCGTGCA
CCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAAACAGGA
25 AGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACT
TTCTCTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATA
TTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTG
CCACCTGACGCTCAAGAAACCATATTATCATGACATTAACTATAAAAAATAGGCGTATC
ACGAGGCCCTTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAACCTCTGACACATGCAG
30 CTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAG
GGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAATATGCGGCATCAGAGCAG
ATTGTACTGAGAGTGCACCATATGGACATATTGTCTGTAAGACGCGGCTACAATTAATAC
ATAACCTTATGTATCATACACATACGATTTAGGTGACACTATAGAATCGACTCTAGAGT
CCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCAT
35 TGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGAATTTCCATTGACGTC
AATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGC
CAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGT
ACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTA
CCATGGTGATCGGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGG
40 GATTTCAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTGGCACCAAAATCAAC
GGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAATGGGCGGTAGGCGTG
TACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTGTGAACCGCGCCAGTCTCCGATAG
ACTGCGTCGCCCGGGTACCCGTATTCCCAATAAAGCCTCTTGCTGTTGCATCCGAATCG
TGGTCTCGCTGTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCACGACGGGGTTC
45 TTTCAATTTGGGGGCTCGTCCGGGATTTGGAGACCCCTGCCAGGGACCACCGACCCACCA
CCGGGAGGTAAGCTGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGTT
TGATGTTATGCGCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCG
TGGTGGAACTGACGAGTTCTGAACACCCGCGCCGCAACCTGGGAGACGTCCAGGGACTT
TGGGGGCCGTTTTTGTGGCCGACCTGAGGAAGGGAGTCGATGTGGAATCCGACCCCGTC
50 AGGATATGTGGTCTGGTAGGAGACGAGAACCTAAACAGTTCCCGCCTCCGTCTGAATT
TTTGTCTTCCGGTTTGGAAACCGAAGCCGCGCTTGTCTGCTGCAGCGCTGCAGCATCGT
TCTGTGTTGTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATAGGGCCAGACTGT
TACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACAA
CCAGTCGGTAGATGTCAAGAAGAGACGTTGG
55

PLTRloxP (SEQ ID No 58)

60 GCTAGCATAACTTCGTATAATGTATGCTATACGAAGTTATTCTAGAGAACCATCAGATGT
TTCCAGGGTGCCCAAGGACCTGAAATGACCCTGTGCCTATTTGAACTAACCAATCAGT

TCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAAC
CCCTCACTCGGGGCGCCAGTCCCTCCGATTGACTGAGTCGCCCCGGGTACCCGTGTATCCAA
TAAACCCCTCTTGCACTTGATCCGACTTGTGGTCTCGCTGTTCTTGGGAGGGTCTCCTC
TGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATTGGGGGCTCGTCCGGGATCGGGA
5 GACCCCTGCCAGGGACCACCGACCCACCGGGAGGTAAGCTGGCTGCCTCGCGCGTT
TCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTC
TGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCTCAGCGGGTGTGGCGGGT
GTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGATATACTGGCTTAATA
TGCGGCATCAGAGCAGATTGTAAGTGCACCATATGCGGTGTGAAATACCGCACAG
10 ATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCT
GCGCTCGGTGTTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTT
ATCCACAGAAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGAAAAGGC
CAGGAACCGTAAAAAGGCCGCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA
GCATCAGAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATA
15 CCAGGCGTTTCCCTCGAAGCTCCCTCGTGGCTCTCTGTTCCGACCCCTGCCGCTTAC
CGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTG
TAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGACGAACCCCC
CGTTTCAGCCGACCGCTGCGCTTATCCGGTAACATCGTCTTGAGTCCAACCCGGTAAG
ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGT
20 AGGCGGTGTACAGAGTCTTGAAGTGGTGGCTAATACGGCTACACTAGAGGACAGT
ATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAGAGTTGGTAGCTCTTG
ATCCGGCAAAACAAACCCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTAC
GCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCTCA
GTGGAACGAAAACTCAGCTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTAC
25 CTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATAGTAAAC
TTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATT
TCGTTTCATCCATAGTTGCCTGACTCCCGTCTGTAGATAACTACGATACGGGAGGGCTT
ACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCAGCTCACCAGCTCCAGATT
ATCAGCAATAAACAGCCAGCCGGAAGGGCGAGCGCAGAAGTGGTCTGCACTTTATC
30 CGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAA
TAGTTTGCACAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTACGCTCGTCTGTTGG
TATGGCTTCATTACGCTCCGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTT
GTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGGCCG
AGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTGATGCCATCCGT
35 AAGATGCTTTTCTGTGACTGGTGAAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCG
GCGACCGAGTTGCTCTTCCCGGCGTCAACACGGGATAATACCGGCCACATAGCAGAAC
TTTAAAGTGCTCATCATTGGAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACC
GCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTT
TACTTTACAGCGTTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAGGG
40 AATAAGGGGACACGGAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAG
CATTTATCAGGGTTATTGCTCATGAGCGGATACATATTGAATGTATTAGAAAAATAA
ACAAATAGGGGTTCCGCGCACATTTCCCGAAAAAGTGCCACCTGACGTCTAAGAAACCAT
TATTATCATGACATTAACTATAAAAAATAGGCGTATCAGAGGCCCTTTCGTCTTCAAGA
ATTCATACCAGATCACCAGAACTGTCTCCAAATGTGTCCCTTCACTCCCAATTC
45 GCGGGCTTCTGCTCTTAGACCACTTACCCTATTCCCACTCACCAGGAGCCAAAGCC
GCGGCCCTTCGCTTCTTTGCTTTTGAAGACCCACCCGTAGGTGGCAA

LTR plasmid (SEQ ID No 59)

GCTAGCTTAAGTAACGCCATTTTGAAGGCATGGAAAAATACATAACTGAGAATAGAGAA
50 GTTCAGATCAAGGTGAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGT
GGTAAGCAGTTCTGCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCC
AAACAGGATATCTGTGGTAAGCAGTTCTTCCCGGCTCAGGGCCAAGAACAGATGGTCC
CCAGATGCGGTCCAGCCCTCAGCAGTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCC
CAAGGACCTGAAATGACCTGTGCCTATTGTAATAACCAATCAGTTTCGCTTCTCGCTT
55 CTGTTTCGCGCGCTTCTGCTCCCGAGCTCAATAAAGAGCCCAACCCCTCACTCGGGG
CGCCAGTCTCCGATTGACTGAGTGCCTCGGCTACCCGTGTATCCAATAAACCCTCTTGC
AGTTGCATCCGACTTGTGGTCTCGCTGTTCTTGGGAGGGTCTCTCTGAGTGATTGACT
ACCCGTGAGCGGGGTCTTTCATTGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCAG
GGACCACCGACCCACCGGGAGGTAAGCTGGCTGCCTCGCGGTTTCGGTGATGACGG

TGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGC
CGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCGCAGC
CATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAACTATGCGGCATCAGAG
CAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGA
5 AATACCGCATCAGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTTCGTT
CGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGTAATACGGTTATCCACAGAATCA
GGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAA
AAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAAT
CGACGCTCAAGTCAGAGGTGGCGAAACCGACAGGACTATAAAGATAACAGGCGTTTTCCC
10 CCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCC
GCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGT
TCGGTGTAGGTCTGCTCCAAGCTGGGCTGTGTGCAGAACCCCCCGTTACGCCCCGAC
CGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG
CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACA
15 GAGTCTTGAAGTGGTGGCTTAACACGGCTACACTAGAAGGACAGTATTTGGTATCTGC
GCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACA
ACCACCGCTGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAA
GGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCTCAGTGGAACGAAAAAC
TCACGTAAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTA
20 AATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGT
TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATA
GTTGCCCTGACTCCCGTCGTGTAGATAAATACGATACGGGAGGGCTTACCATCTGGCCCC
AGTGCTGCAATGATACCGCGAGACCCACGCTACCGGCTCCAGATTATCAGCAATAAAC
CAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAG
25 TCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCCGCAAGTTAATAGTTTGCGCAAC
GTTGTTGCCATTGCTGCAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTC
AGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCG
GTTAGCTCCTTCGGTCCCGATCGTTGTGAGAAGTAAGTTGGCCGCGAGTGTATCACTC
ATGGTTATGGCAGCACTGCATAATTCTCTTACTGTATGCCATCCGTAAGATGCTTTTCT
30 GTGACTGGTGAAGTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGC
TCTTGCCCGGCGTCAACACGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGCTC
ATCATTGGAACGTTCTTCGGGGCGAAACTCTCAAGGATCTTACCGCTGTTGAGATCC
AGTTCGATGTAACCCACTCGTGACCCCAACTGATCTTCAGCATCTTTTACTTTTACCAGC
GTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACA
35 CGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGT
TATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTT
CCGCGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACATTATTATCATGACA
TTAACCTATAAAAATAGGCGTATCAGGAGGCCCTTTCGTCTTCAAGAATTATACAGAT
CACCAGAACTGTCTCCAAATGTGTCCCCCTCACACTCCCAATTCGCGGGCTTCTGCC
40 TCTTAGACCACTTACCCTATTCCCACTACCGGAGCCAAAGCCGCGGCCCTTCCGT
TTCTTTGCTTTTGAAAGACCCACCCGTAGGTGGCAA

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03837

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/867 C12N15/90 C12N5/10 C12N7/01 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>VANIN E. F. ET AL.: "Development of high-titer retroviral producer cell lines by using Cre-mediated recombination." JOURNAL OF VIROLOGY, vol. 71, no. 10, 1997, pages 7820-7826, XP002161355 ISSN: 0022-538X cited in the application the whole document</p> <p style="text-align: center;">— — — — — — / —</p>	1-51

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *8* document member of the same patent family

Date of the actual completion of the international search

26 February 2001

Date of mailing of the international search report

13/03/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03837

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KARREMAN S. ET AL.: "ON THE USE OF DOUBLE FLP RECOGNITION TARGETS (FRTS) IN THE LTR OF RETROVIRUSES FOR THE CONSTRUCTION OF HIGH PRODUCER CELL LINES" NUCLEIC ACIDS RESEARCH, vol. 24, no. 9, 1 May 1996 (1996-05-01), pages 1616-1624, XP000616161 ISSN: 0305-1048 cited in the application the whole document	1-51
A	----- IWAKUMA T. ET AL.: "SELF-INACTIVATING LENITVIRAL VECTORS WITH U3 AND U5 MODIFICATIONS" VIROLOGY, vol. 261, no. 1, 15 August 1999 (1999-08-15), pages 120-132, XP000882897 ISSN: 0042-6822 the whole document	1-51
A	----- BOAST K. ET AL.: "CHARACTERIZATION OF PHYSIOLOGICALLY REGULATED VECTORS OF THE TREATMENT OF ISCHEMIC DISEASE" HUMAN GENE THERAPY, vol. 10, no. 13, 1 September 1999 (1999-09-01), pages 2197-2208, XP000876772 ISSN: 1043-0342 the whole document	1-51

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 45-48

Said claims 45-48 could not be searched because they were not present in the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.